Exhibit 25

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Risk factors for ovarian cancer: a case-control study

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Summary A hospital-based case-control study of ovarian cancer was conducted in London and Oxford between October 1978 and February 1983. Menstrual characteristics, reproductive and contraceptive history and history of exposure to various environmental factors were compared between 235 women with histologically diagnosed epithelial ovarian cancer and 451 controls. High gravidity, hysterectomy, female sterilisation and oral contraceptive use were associated with a reduced risk of ovarian cancer. Infertility and late age at menopause were associated with an increase in risk. While these factors were related, they were each found to be independently associated with ovarian cancer risk after adjusting for the effect of the other factors.

While results from recent case-control studies have consistently shown that multiparity and oral contraceptive use are associated with a reduced risk of ovarian cancer, the association of the cancer with other reproductive, hormonal and related factors such as age at menopause, history of hysterectomy or use of oestrogen replacement therapy is less clear. We have conducted a hospital-based case-control study in London and Oxford which was designed to investigate the independent contributions of reproductive history and contraceptive use to ovarian cancer risk. In particular, it was planned to attempt to segregate out the effect on risk of infertility from that of voluntary limitation of family size. The association between ovarian cancer and other possible aetiological agents was also examined.

Subjects and methods

Between October 1978 and February 1983 five interviewers identified and questioned women with a diagnosis of ovarian cancer and women selected as controls at 13 hospitals in London and two in Oxford. A standard questionnaire was used to obtain information on reproductive and menstrual history and on exposure to various substances such as exogenous oestrogens, cigarettes and talc. A month by month record was made of the specific contraceptive methods used by each woman between the ages of 16 and 45 years, or, if under 45 years, up to the time of diagnosis (cases) or interview (controls). The methods were classified as sheaths, diaphragms, intrauterine devices, oral contraceptives or 'other methods' (spermicides, rhythm and coitus interruptus). Women who reported using a contraceptive diaphragm were asked if they had stored it in talc. Also recorded were months during which a woman was not using contraception due to sexual abstinence, pregnancy, menopause or because she or her partner had been sterilised. The other months when a woman reported using no method of contraception although sexually active have been classified as months of 'unprotected intercourse'. The total duration of use of each contraceptive method, of any contraceptive method, of unprotected intercourse and of pregnancy were computed for each woman.

The study was confined to women aged less than 65 years whose diagnosis of ovarian cancer had been made within two years of interview. A total of 280 cases were interviewed and pathological specimens were histologically classified by Professor C. Hudson and Dr M. Curling from St Bartholomews Hospital. A total of 235 women with epithelial ovarian cancer were included in the analyses. For these women, the tumour type was described as serous in 101 (43%) cases, mucinous in 38 (15%) cases, endometrioid in 52 (22%) cases and clear cell in 12 (5%) cases. Mixed and undifferentiated types of epithelial tumours accounted for the remaining 32 (14%) cases. Excluded from the analyses were nine women with a non-epithelial ovarian neoplasm, 11 with a primary tumour in an unknown site outside the ovary, 21 with a primary tumour in an unknown site although one consistent with an ovarian origin, one with a benign tumour and three for whom pathology material could not be obtained.

For each case it was planned to select two age-matched controls from women being treated in the same hospital. Women with bilateral oophorectomy were excluded from the control group as were women admitted with conditions that have been related to reproductive history or oral contraceptive use (all circulatory and gynaecological diseases, gallbladder and thyroid diseases, rheumatoid arthritis, malignant disease of the breast, uterus and bladder, and melanoma).

It proved logistically impossible to select two age-matched controls for each case from the same hospital and it was decided merely to ensure that the age distribution of the controls was approximately the same as that of the cases. For 63 cases recruited from a London hospital where only cancer patients are treated, controls were selected from other London hospitals. For these reasons, the data were analysed using an unmatched approach with adjustments being made to relative risk estimates for age and socio-economic status. A total of 451 controls have been included in the analyses. The admission diagnoses for these patients were gastrointestinal disease (105), bone or joint disease (70), respiratory disease (39), renal or other urinary disease (35), neurological disease (30), fractures or other injuries (28), skin or subcutaneous tissue disease (17), malignant neoplasms of the digestive organs (15) and bone or skin (2), benign neoplasms of the digestive organs (4) respiratory system (4) and other sites (8) and various other conditions and symptoms (94). This final category included patients with haemorrhoids (15) and those with symptoms relating to the respiratory system (10), gastrointestinal tract (20) and urinary system (10).

Maximum likelihood estimates of relative risk (RR) together with their 95% confidence interval (95% CI) and tests for trend where appropriate were computed by multiple logistic regression techniques (Breslow & Day, 1980) using the GLIM statistical package (Baker & Nelder, 1978). All relative risks have been adjusted for age in 5-year strata (20-24, 25-29, . . . 60-64) and for social class in six categories (I,II,III non-manual, III manual, IV and V). Age of the cases was taken as age at diagnosis of ovarian cancer and of the controls as age at interview. Social class was based on occupation (Office of Population Censuses and Surveys, 1970) using husband's occupation for ever married women and own occupation for those who had never married. Other relative risk adjustments and tests for trend have been made with the exposures as continuous variables. When the data were examined by place of interview (London or Oxford), there were no notable differences in the risk estimates

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associated with the major variables of interest. The relative risks have not, therefore, been stratified by place of interview. The terms nulligravid and gravid have been used to denote, respectively, women who have never knowingly conceived and women who have had at least one pregnancy. Parity has been defined as number of live and still births.

Results

The age distributions of the cases and controls are shown in Table I. The average age of the cases was slightly higher than that of the controls. There was an excess of cases in social classes I, II, and III non-manual (58%) as compared to controls (43%) ($P = 0.05$) and, because of this, all relative risks have been adjusted for social class as well as age. Table II shows the relative risks for ovarian cancer associated with various aspects of pregnancy history. Nulligravid women had a higher risk of ovarian cancer than gravid women (RR = 1.7, 95% CI 1.1–2.6). The relative risks were elevated both in nulligravid women who had been sexually active and in those who had not, although significantly so only for the sexually active. Among those who had ever been pregnant, the relative risks decreased as the number of pregnancies increased, (χ^2 (trend) = 4.3, $P < 0.05$). Similarly, among parous women, the higher the parity the lower the relative risks (χ^2 (trend) = 3.9, $P < 0.05$). After adjusting for parity, the relative risks associated with successive numbers of incomplete pregnancies (spontaneous and induced abortions) also decreased although the trend was not statistically significant (χ^2 (trend) = 0.5). Women having their first pregnancy after the age of 35 years had a significantly higher risk of ovarian cancer than women with a first pregnancy before the age of 20 years. Their risk was also higher than that for nulligravid women. There was, however, no marked nor significant trend of increasing risk the later the age at first pregnancy (χ^2 (trend) = 1.0). Analyses by age at first livebirth gave similar findings. After adjustment for number of livebirths, women who had breastfed for more than two years in total had over three times the risk of ovarian cancer compared to women who had never breastfed ($P < 0.05$) but overall, there was no significant trend the longer the duration of lactation.

Analyses of infertility and subfertility as risk factors for ovarian cancer were restricted to the 213 (91%) cases and 240 (93%) controls who reported that they had ever been sexually active. Among these women, 30 (14%) with ovarian cancer and 34 (8%) controls reported, when so questioned, that they had had problems in becoming pregnant and, of these, 16 cases and 12 controls had never conceived. Analysis of the data on contraceptive use suggested that there were other women who might have been infertile or subfertile. Although sexually active, they had used contraception infrequently or not at all and had had few or no pregnancies. For all women who had ever been sexually active, the risk of ovarian cancer increased with increasing duration of unprotected intercourse after adjustment for gravidity (χ^2 (trend) = 10.2, $P < 0.01$). The effect was most marked among nulligravid women who reported more than 10 years of unprotected intercourse. Their risk was over six times that of nulligravid women who reported less than three months of unprotected intercourse (Table III). Among gravid women, those reporting over 10 years of unprotected intercourse had a higher risk than other gravid women. There was no significant trend in risk associated with the duration of use of any contraception (χ^2 (trend) = 1.2) although sexually active nulligravid women who had never used any method of contraception had about twice the risk of ovarian cancer compared to all other sexually active women (Table IV).

Of the specific methods of contraception studied, ever having used oral contraception and having been sterilised were associated with a statistically significantly reduced risk of ovarian cancer, while no method was associated with a significantly elevated risk (Table V). As only three cases had been sterilised it was not possible to assess whether age at sterilisation influenced the risk of ovarian cancer. Table VI shows detailed analyses of the relative risks associated with oral

Table I Age distribution and average age of cases and controls

Age (years)	*Cases (%)*	*Controls (%)*
20–34	13 (5.5)	33 (7.3)
35–44	27 (11.5)	75 (16.6)
45–54	87 (37.0)	156 (34.6)
55–64	108 (46.0)	187 (41.5)
Total	235	451
Average age (years)	52.4	51.4

Table II Relative risks for ovarian cancer associated with pregnancy history

Variable	*Cases*	*Controls*	*RR*	*(95% CI)*
Gravidity[b]				
Gravid	176	376	1.0[a]	
Nulligravid	59	74	1.7	(1.1–2.6)
Nulligravid and ever sexually active	37	44	1.9	(1.1–3.1)
Nulligravid and never sexually active	22	30	1.5	(0.8–2.6)
Number of pregnancies				
0	59	74	1.0[a]	
1	43	71	0.8	(0.4–1.3)
2	63	107	0.7	(0.4–1.1)
3	37	98	0.5	(0.3–0.8)
4	13	41	0.4	(0.2–0.8)
⩾5	20	59	0.4	(0.2–0.8)
	χ^2 for trend = 4.3 $P<0.05$ (gravid women only)			
Estimated reduction in relative risk associated with each pregnancy			0.86	(0.78–0.94)
Parity[b]				
0	66	87	1.0[a]	
1	48	84	0.7	(0.4–1.2)
2	61	127	0.6	(0.4–1.0)
3	40	88	0.6	(0.3–1.0)
4	12	30	0.5	(0.2–1.0)
⩾5	8	34	0.3	(0.1–0.7)
	χ^2 for trend = 3.9 $P<0.05$ (parous women only)			
Estimated reduction in relative risk associated with each birth			0.84	(0.75–0.94)
No. of incomplete pregnancies[b,c]				
0	185	330	1.0[a]	
1	39	83	0.9	(0.6–1.4)
2	7	18	0.7	(0.3–1.8)
⩾3	4	19	0.6	(0.2–1.7)
	χ^2 for trend = 0.5			
Estimated reduction in relative risk associated with each incomplete pregnancy			0.92	(0.75–1.13)
Age at first pregnancy (years)[d]				
15–19	26	65	1.0[a]	
20–24	73	182	0.9	(0.5–1.5)
25–29	49	96	1.2	(0.7–2.2)
30–34	17	29	1.2	(0.8–2.7)
⩾35	9	4	4.1	(1.1–15.1)
Nulligravid	59	74	2.0	(1.1–3.7)
	χ^2 for trend = 1.0 (gravid women only)			
Months of lactation[e]				
None	44	107	1.0[a]	
⩽6	66	124	1.3	(0.8–2.2)
7–12	29	80	0.9	(0.5–1.6)
13–18	13	29	1.2	(0.5–2.5)
19–24	5	7	2.1	(0.7–6.7)
⩾25	12	15	3.4	(1.1–10.8)
	χ^2 for trend = 1.8			

All relative risks adjusted for age and social class. [a]Reference category. [b]Data missing for 1 control. [c]Relative risks adjusted for parity. [d]Data missing for 2 cases and 1 control. [e]Women with livebirths only. Relative risks adjusted for number of live births.

Table III Relative risks for ovarian cancer associated with duration of unprotected intercourse by gravidity

	Cases	*Controls*	*RR*	*(95% CI)*
Nulligravid women				
Duration of unprotected intercourse (months)[b]				
⩽3				
4–60	12	26	1.0[a]	(0.4–6.5)
61–120	4	6	1.5	(0.1–7.8)
>120	1	4	0.7	(2.1–20.4)
	20	8	6.5	
χ^2 for trend = 11.2 $P<0.001$				
Gravid women				
Duration of unprotected intercourse (months)[b]				
⩽3				
4–60	78	176	1.1	(0.5–2.4)
61–120	51	113	1.1	(0.5–2.6)
>120	10	27	1.1	(0.4–3.2)
	37	60	1.6	(0.7–4.0)
χ^2 for trend = 2.6				

Sexually active women only. Relative risks adjusted for age and social class. [a]Reference category. [b]Time when sexually active and at risk of pregnancy but using no contraception.

Table IV Relative risks for ovarian cancer associated with duration of use of contraception by gravidity

	Cases	*Controls*	*RR*	*(95% CI)*
Nulligravid women				
Duration of use of contraception				
Never used	15	10	1.0[a]	
<10 years	14	21	0.5	(0.1–1.7)
10–20 years	6	9	0.5	(0.1–2.5)
>20 years	2	4	0.4	(0.1–3.2)
χ^2 for trend = 0.9				
Gravid women				
Duration of use of contraception				
Never used	32	47	0.4	(0.2–1.1)
<10 years	25	56	0.3	(0.1–1.0)
10–20 years	55	147	0.4	(0.1–1.2)
>20 years	64	126	0.5	(0.2–1.5)
χ^2 for trend = 0.3				

Sexually active women only. Relative risks adjusted for age, social class and duration of unprotected intercourse. [a]Reference category.

Table V Relative risks for ovarian cancer associated with the use of different methods of contraception

Method of contraception		*Cases*	*Controls*	*RR*	*(95% CI)*
Sheath	Never used	108	205	1.0[a]	
	Ever used	105	215	1.1	(0.8–1.7)
Diaphragm	Never used	178	329	1.0[a]	
	Ever used	35	91	0.7	(0.4–1.1)
Intrauterine device	Never used	201	383	1.0[a]	
	Ever used	12	37	0.8	(0.4–1.7)
Oral contraception	Never used	178	306	1.0[a]	
	Ever used	35	114	0.5	(0.3–0.9)
Partner with vasectomy	No	203	404	1.0[a]	
	Yes	10	16	2.1	(0.9–4.9)
Female sterilisation	No	210	375	1.0[a]	
	Yes	3	45	0.2	(0.1–0.6)
Other methods[b]	Never Used	156	292	1.0[a]	
	Ever used	57	128	1.1	(0.7–1.7)

Sexually active women only. Relative risks adjusted for age, social class, gravidity and duration of unprotected intercourse. [a]Reference category. [b]Use of spermicides, rhythm or coitus interruptus.

contraceptive use. The risks decreased as duration of use increased although, among those who had ever used such contraceptives, the trend was not significant (χ^2 (trend) = 1.2). Whatever their age at first use, women who used oral contraceptives had a lower risk of ovarian cancer than those who had never used them, the risk being lowest in those who had first used oral contraceptives under the age of 25 years. The risk of developing ovarian cancer did not increase as time since discontinuing use increased. Women who had stopped using oral contraceptives more than ten years previously had a statistically significant reduced risk of 0.3 compared to women who had never used them. Women both under the age and over the age of 40 years had a reduced risk of ovarian cancer associated with oral contraceptive use, but the reduction was greater in the younger women. Gravid and nulligravid women who had used oral contraceptives had a reduced risk of ovarian cancer.

Table VII shows the relative risks associated with age at menarche and age at natural menopause. There was no trend in risk with age at menarche (χ^2 (trend) = 0.03). In contrast, risk increased the later the age at natural menopause (χ^2 (trend) = 7.1, $P<0.01$). Women having their menopause at the age of 50 years or later had nearly three times the risk of women who were menopausal before the age of 45 years. The risks and trend associated with age at menopause were similar irrespective of whether they were adjusted for age in five year or one year strata.

Table VI Relative risks for ovarian cancer associated with oral contraceptive (OC) use

	Cases	*Controls*	*RR*	*(95% CI)*
Duration of OC use (years)				
Never used	178	306	1.0[a]	
<5	24	70	0.6	(0.3–1.0)
5–10	10	29	0.6	(0.2–1.4)
>10	1	15	0.1	(0.01–1.0)
χ^2 for trend within users = 1.2				
Age at first OC use (years)				
Never used	178	306	1.0[a]	
<25	6	39	0.1	(0.04–0.5)
25–29	6	17	0.6	(0.2–2.0)
30–34	11	27	0.7	(0.3–1.6)
⩾35	12	31	0.7	(0.4–1.5)
χ^2 for trend within users = 5.9, $P<0.05$				
Time since discontinuing OC use (years)				
Never used	178	306	1.0[a]	
Current users	6	19	0.5	(0.2–1.5)
<5	12	24	0.8	(0.4–1.9)
5–10	9	25	0.8	(0.3–1.9)
>10	8	46	0.3	(0.1–0.7)
χ^2 for trend within users = 2.6				
Age (years)				
<40				
OC use				
Never used	11	11	1.0[a]	
Ever used	9	35	0.2	(0.1–0.9)
⩾40				
OC use				
Never used	167	295	1.0[a]	
Ever used	26	79	0.7	(0.4–1.2)
Gravidity				
Gravid women[b]				
OC use				
Never used	149	280	1.0[a]	
Ever used	27	96	0.5	(0.3–0.9)
Nulligravid women				
OC use				
Never used	29	26	1.0[a]	
Ever used	8	18	0.3	(0.05–2.8)

Sexually active women only. Relative risks adjusted for age, social class, gravidity and duration of unprotected intercourse. [a]Reference category. [b]Relative risks adjusted for gravidity.

Table VII Relative risks for ovarian cancer associated with age at menarche and age at natural menopause

	Cases	*Controls*	*RR*	*(95% CI)*
Age at menarche (years)[b]				
>14	97	197	1.0[a]	
12–13	89	185	0.9	(0.6–1.3)
<12	46	66	1.3	(0.8–2.1)
	χ^2 for trend = 0.03			
Age at natural menopause (years)[c]				
<45	10	34	1.0[a]	
45–49	47	77	2.0	(0.9–4.7)
>50	84	99	2.5	(1.1–5.8)
	χ^2 for trend = 7.1 $P<0.01$			

Relative risks adjusted for age and social class. [a]Reference category. [b]Data on age at menarche missing for 3 cases and 3 controls. [c]Data on age at menopause missing for 2 cases and 1 control.

Women who reported hysterectomy, with or without unilateral oophorectomy, had a much reduced risk (Table VIII). Since there were only 10 women with ovarian cancer who had had a hysterectomy it was not possible to assess the effect of age at hysterectomy on ovarian cancer risk.

Total duration of ovulation was estimated as the months from menarche to diagnosis (cases) or interview (controls), or to menopause, whichever came first, minus the total months of anovulation due to pregnancy and oral contraceptive use. Women who reported a hysterectomy were excluded from these analyses as it was unknown if or when they had stopped ovulating. For all women combined, there was a strong trend of increasing risk the longer the duration of ovulation (χ^2 (trend) = 17.8, $P<0.001$) (Table IX). In separate analyses by menopausal status, there was no significant effect of duration of ovulation after adjustment for the 'anovulatory' factors used to estimate that exposure, namely, months of pregnancy and oral contraceptive use and age at menopause for post-menopausal women and months of pregnancy and oral contraceptive use for premenopausal women. Duration of ovulation is very sensitive to age but the risks and trends were virtually unaffected when adjusted for age in one year rather than five year strata.

Five (2%) cases and 29 (6%) controls reported having taken hormone pills as a pregnancy test and five (2%) cases and 13 (3%) controls had been given hormones to prevent miscarriage. For all post-menopausal women, there was a small but non-significantly increased risk of ovarian cancer associated with ever having recieved hormone replacement therapy (Table X). The excess was confined to women who had reported a hysterectomy who had an 11-fold risk. The cases did not report more severe menopausal symptoms. Among the hormone treated women with ovarian cancer, 23% had endometrioid or clear cell tumours compared to 38% in the untreated women.

The reproductive and related factors found to be statistically significantly related to ovarian cancer risk (gravidity, duration of unprotected intercourse, use of oral contraception, having been sterilised, age at natural menopause and having had a hysterectomy) are not independent and we also computed the relative risks associated with each factor after adjusting for the others (Table XI). As sterilisation is often a consequence of high parity, the risks associated with gravidity were not adjusted for sterilisation as this was considered to be overadjustment. In this study, 40% of the sterilised women had five or more children compared with 9% of the unsterilised women. Each of the variables remained statistically significantly related to ovarian cancer risk, suggesting that each may be independently associated with the risk of developing ovarian cancer.

There was no significant difference between the percentage of cases (53%) and controls (57%) who had ever smoked cigarettes. No cases or controls reported having worked with asbestos. No cases but three controls reported a radiation-induced menopause.

Women who reported using talc more than once a week or daily had higher risks of ovarian cancer than women who reported less frequent use (Table XII). Although the relative risk of 2.0 associated with weekly use was statistically significant

Table VIII Relative risks for ovarian cancer associated with reported history of hysterectomy and/or unilateral oophorectomy

	Cases	*Controls*	*RR*	*(95% CI)*
Reported womb intact	220	370	1.0[a]	
Reported unilateral oophorectomy by no hysterectomy	5	9	0.9	(0.4–2.1)
Reported hysterectomy but conserved ovaries	8	62	0.2	(0.1–0.4)
Reported hysterectomy and unilateral oophorectomy	2	10	0.4	(0.1–1.1)

Relative risks adjusted for age and social class. [a]Reference category.

Table IX Relative risks for ovarian cancer associated with duration of ovulation

	Duration of ovulation (years)	*Cases*	*Controls*	*Relative risks adjusted for age and social class*	*Relative risks adjusted for age, social class and duration of anovulation*	*Relative risks adjusted for age, social class, duration of anovulation and age at menopause*
All women[b]						
	<30	59	163	1.0[a]		
	30–34	73	106	2.0		
	35–39	68	92	2.0		
	>40	19	13	4.3		
				χ^2 for trend = 17.8 $P<0.001$		
Post-menopausal women						
	<30	14	53	1.0[a]	1.0[a]	1.0[a]
	30–34	54	81	2.4	2.1	0.9
	35–39	58	72	2.4	1.9	0.6
	>40	14	10	5.0	4.0	0.7
				χ^2 for trend = 12.3 $P<0.001$	7.7 $P<0.01$	0.5
Premenopausal women						
	<30	45	110	1.0[a]	1.0[a]	
	30–34	19	25	1.5	1.1	
	35–39	10	20	1.3	0.9	
	>40	5	3	3.2	1.9	
				χ^2 for trend = 4.4 $P<0.05$	0.6	

Women reporting a hysterectomy excluded: [a]Reference category. [b]Data missing for 6 cases and 4 controls. [c]Total months of pregnancy and oral contraceptive use.

Table X Relative risks for ovarian cancer associated with the use of hormone replacement therapy for menopausal symptoms

	Cases	*Controls*	*RR*	*(95% CI)*
All post-menopausal women				
Use of hormone replacement therapy				
No	122	249	1.0[a]	
Yes	34	44	1.5	(0.9–2.6)
Women reporting hysterectomy				
Use of hormone replacement therapy				
No	5	62	1.0[a]	
Yes	5	10	10.9	(1.7–69.0)
Post-menopausal women other than those reporting hysterectomy				
Use of hormone replacement therapy				
No	177	187	1.0[a]	
Yes	29	34	1.2	(0.7–2.3)

Post-menopausal women only. Relative risks adjusted for age and social class. [a]Reference category.

Table XI Relative risks associated with the factors found to be significantly related to ovarian cancer

Factor	*RR*	*(95% CI)*	χ^2 test for trend (1 d.f.)
Gravidity[b,c]			
0	1.0[a]		
1	0.8	(0.4 –1.5)	
2	0.8	(0.4 –1.4)	
3	0.6	(0.3 –1.1)	
4	0.4	(0.2 –1.0)	
⩾5	0.5	(0.2 –1.0)	6.5 $P<0.05$
Unprotected intercourse (months)[b]			
<3	1.0[a]		
4–60	1.3	(0.8 –2.0)	
61–120	1.1	(0.5 –2.5)	
>120	1.9	(1.2 –3.2)	7.8 $P<0.05$
Oral contraceptive use[b]			
Never used	1.0[a]		
⩽5 years	0.6	(0.3 –1.1)	
6–10 years	0.6	(0.3 –1.4)	
>10 years	0.1	(0.02–1.1)	4.6 $P<0.05$
Ever sterilized[b]	0.2	(0.05–0.6)*	
Age at natural menopause[d]			
<45 years	1.0[a]		
45–49 years	1.9	(0.8 –4.5)	8.2 $P<0.01$
⩾50 years	2.6	(1.1 –6.1)	
Ever reported hysterectomy[b]	0.2	(0.1 –0.5)*	

The relative risks associated with each factor have been adjusted for age, social class and all the other factors in the table. [a]Reference category. [b]Sexually active women only. [c]Relative risks not adjusted for sterilization, see text for details. [d]Women reporting natural menopause only. *$P<0.001$.

Table XII Relative risks for ovarian cancer associated with reported frequency of talc use in the genital area

	Cases	*Controls*	*RR*	*(95% CI)*
Reported frequency of talc use[b]				
Never	76	178	1.0[a]	
Rarely	6	16	0.9	(0.3–2.4)
Monthly	7	24	0.7	(0.3–1.8)
Weekly	57	77	2.0	(1.3–3.4)
Daily	71	139	1.3	(0.8–1.9)
	χ^2 for trend = 3.80, $P=0.05$			

Relative risks adjusted for age and social class. [a]Reference category. [b]Data missing for 18 (8%) cases and 17 (4%) controls as questions on talc use introduced three months after study began.

($P=0.007$), there was no consistent trend of increasing risk with increasing frequency of talc use (χ^2 (trend) = 3.80, $P=0.05$). There was no significant difference between the percentages of cases (86%) and controls (81%) who had used and kept their diaphragm in talc.

Discussion

As in most previously reported studies (Booth & Beral, 1985) we found that nulligravid women had an increased risk of ovarian cancer and that risk decreased as the number of pregnancies increased. We also found that the greater the number of incomplete pregnancies the lower the risk, although the trend was not significant. Most other studies have not investigated if women of low gravidity have an increased risk of ovarian cancer because of reduced fertility or because of voluntary limitation of family size, although Joly *et al.* (1974), McGowan *et al.* (1979) and Nasca *et al.* (1984) found a higher risk in women who had tried to conceive but had failed. Our findings also suggest that infertility is a risk factor for ovarian cancer. Women who had not conceived but had been sexually active for more than 10 years without using contraception had about six times the risk of all other women. Approximately half these women had undergone investigations for infertility. For only five cases and one control was the cause of their infertility determined. Thus, it is not possible to assess whether this high risk group had normal or impaired ovarian function. Subfertility may also be associated with ovarian cancer. Gravid women reporting over 10 years of unprotected intercourse had a 50% higher risk than other gravid women, but this increase was not statistically significant.

Age at first pregnancy was not found to be associated with ovarian cancer risk although women having a first pregnancy after the age of 35 years had a higher risk compared to women having a first pregnancy at earlier ages and to nulligravid women. Since subfertility might be a risk factor for ovarian cancer, the relative risks associated with age at first pregnancy were also adjusted for duration of unprotected intercourse. The raised risk for women with a first pregnancy after 35 years persisted (RR = 3.9, 95% CI 1.1–14.2). Results from other studies regarding the risk for women having a first child at relatively older ages are inconclusive, some finding no association (Newhouse *et al.*, 1977; Casagrande *et al.*, 1979; Cramer *et al.*, 1983; Lesher *et al.*, 1985), others an increased risk (Joly *et al.*, 1974; McGowan *et al.*, 1979; Hildreth *et al.*, 1981; Franceschi *et al.*, 1982). Only Franceschi *et al.* (1982) found the increased risk to be statistically significant and independent of parity.

Overall, there was no association between use of any contraception and ovarian cancer. Of the specific methods studied, female sterilisation and use of oral contraception were associated with a significant reduction in risk and no method was associated with a significant increase in risk. The associations remained after adjusting for gravidity and duration of unprotected intercourse, the measure used to indicate infertility. While few studies have examined the association between female sterilisation and ovarian cancer, the relation between oral contraceptives and ovarian cancer has been demonstrated in many studies (Booth & Beral, 1985). Like others, we demonstrated that the longer oral contraceptives had been used, the lower the risk. Our findings also suggested that the earlier the age at first use the lower the risk and that the protective effect of oral contraceptives persists after their use is stopped.

It has been suggested that inhibition of ovulation, as induced by pregnancy and oral contraceptives, is the factor which protects against ovarian cancer (Fathalla, 1971). If so, postpartum anovulation associated with lactation might also be expected to be protective. We found no evidence that the longer a woman had breastfed the lower her risk of ovarian cancer. Indeed, the highest risk was found in those who had breastfed longest. Results from other studies are contradic-

tory (Cramer *et al.*, 1983; Mori *et al.*, 1984; Risch *et al.*, 1983; Cancer and Steroid Hormone Study, 1987).

Our finding that age at menarche was not associated with risk is consistent with results from most other studies (Casagrande *et al.*, 1979; McGowan *et al.*, 1979; Hildreth *et al.*, 1981; Franceschi *et al.*, 1982). Age at natural menopause, however, was strongly related to risk. While Hildreth *et al.* (1981), Franceschi *et al.* (1982) and Tzonou *et al.* (1984) also demonstrated that the later the age at menopause the greater the risk, other studies have found no association (West, 1966; Newhouse *et al.*, 1977; Annegers *et al.*, 1979; McGowan *et al.*, 1979; Cramer *et al.*, 1983).

A lower frequency of hysterectomy, of unilateral oophorectomy, or of both among cases compared to controls has also been reported from several other studies (Wynder *et al.*, 1969; Joly *et al.*, 1974; Annegers *et al.*, 1979; McGowan *et al.*, 1979; Franceschi *et al.*, 1982; Cramer *et al.*, 1983). As these studies were case–control in design, there may have been some misclassification of controls who, rather than having a hysterectomy with ovarian conservation, actually had a hysterectomy with bilateral oophorectomy. Another explanation for the findings might be that if at hysterectomy a woman's ovaries look diseased, it is likely that they are removed. If the diseased ovaries were precancerous, those women who might otherwise have developed ovarian cancer do not. Following hysterectomy with ovarian conservation, reduced ovarian function or ovarian failure occurs in a proportion of women, due possibly to the blood supply to the ovaries being compromised (Beavis *et al.*, 1969; Ellsworth *et al.*, 1983). Female sterilisation was also associated with a low risk of ovarian cancer. Neil *et al.* (1975) have suggested that the menstrual disturbance that many women experience after sterilisation may reflect changed ovarian function due to damage to the vascular supply to the ovaries. If both hysterectomy and female sterilisation can indirectly affect ovarian function then both procedures could also influence the risk of ovarian cancer.

Recent investigators have shown that the longer a woman ovulates the greater her risk of ovarian cancer (Casagrande *et al.*, 1979; Hildreth *et al.*, 1981; Franceschi *et al.*, 1982; Wu *et al.*, 1988). We also found that risk increased the longer the duration of ovulation. Duration of ovulation is, however, highly correlated with the 'anovulatory' factors used to estimate the exposure. In an attempt to determine whether duration of ovulation had an effect over and above that expressed by its relation with these factors, the risks and trends were adjusted for duration of anovulation due to pregnancy and oral contraceptive use and, where appropriate, for age at menopause. The significance of the effect disappeared. We conclude that it is not possible to determine from these data whether it is the above factors which inhibit ovulation that prevent ovarian cancer, whether repeated ovulations promote it, or whether a combination of both is acting.

Our finding of no overall relationship between hormone replacement therapy and ovarian cancer supports those of other investigators (Newhouse *et al.*, 1977; Hildreth *et al.*, 1981; Weiss *et al.*, 1982). An increased risk associated with the therapy was found among women who reported hysterectomy, but the finding was based on very few cases and may have been due to chance. We did not find an increased risk associated with oestrogen therapy for any particular tumour types as suggested by Cramer *et al.* (1981) and Weiss *et al.* (1982).

The evidence linking talc with ovarian cancer is controversial (Anonymous, 1977; Roe, 1979; Longo & Young 1979; Cramer *et al.*, 1982; Hartge *et al.*, 1983). In this study, women who reported talc use in the genital area more than once a week or daily had higher risks of ovarian cancer than women who used talc less frequently. The women were not asked how long they had been using talc. It is possible that because of their symptoms or disease-related pelvic examinations, the frequency of current talc use by the cases may not have reflected their frequency of past use. Since these and other results (Cramer *et al.*, 1982; Hartge *et al.*, 1983) are insufficient to reject an association, further work is needed on the relation between genital use of talc and ovarian cancer.

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References

ANNEGERS, J.F., STROM, H., DECKER, D.G., DOCKERTY, M.B. & O'FALLON, W.M. (1979). Ovarian cancer: incidence and case control study. *Cancer*, **43**, 723.

ANONYMOUS (1977). Cosmetic talc powder. Editorial. *Lancet*, **i**, 1348.

BAKER, R.J. & NELDER, J.A. (1978). *The GLIM System Release 3*. Royal Statistical Society: Oxford.

BEAVIS, E.L.G., BROWN, J.B. & SMITH, M.A. (1969). Ovarian function after hysterectomy with conservation of the ovaries in premenopausal women. *J. Obstet. Gynaecol. Br. Cwlth*, **76**, 969.

BOOTH, M. & BERAL, V. (1985). The epidemiology of ovarian cancer. In *Ovarian Cancer*, Hudson, C.N. (ed), p. 22. Oxford University Press: Oxford.

BRESLOW, N.E. & DAY, N.E. (1980). *Statistical Methods in Cancer Research, Vol. 1. The Analysis of Case–control Studies*. IARC Scientific Publications No. 32. IARC: Lyon.

CANCER AND STEROID HORMONE STUDY OF THE CENTRES FOR DISEASE CONTROL AND THE NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT (1987). The reduction in risk of ovarian cancer associated with oral-contraceptive use. *N. Engl. J. Med.*, **316**, 650.

CASAGRANDE, J.T., PIKE, M.C., ROSS, R.K., LOUIE, E.W., ROY, S. & HENDERSON, B.E. (1979). 'Incessant ovulation' and ovarian cancer. *Lancet*, **ii**, 170.

CRAMER, D.W., DEVESA, S.S. & WELCH, W.R. (1981). Trends in the incidence of endometrioid and clear cell cancers of the ovary in the United States. *Am. J. Epidemiol.*, **114**, 201.

CRAMER, D.W., WELCH, W.R., SCULLY, R.E. & WOJCIECHOWSKI, C.A. (1982). Ovarian cancer and talc: a case-control study. *Cancer*, **50**, 372.

CRAMER, D.W., HUTCHISON, G.B., WELCH, W.R., SCULLY, R.E. & RYAN, K.J. (1983). Determinants of ovarian cancer risk. I. Reproductive experiences and family history. *J. Natl Cancer Inst.*, **71**, 711.

ELLSWORTH, L.R., ALLEN, H.H. & NISKER, J.A. (1983). Ovarian function after radical hysterectomy for Stage 1B carcinoma of cervix. *Am. J. Obstet. Gynecol.*, **145**, 185.

FATHALLA, M.F. (1971). Incessant ovulation–a factor in ovarian neoplasia? *Lancet*, **ii**, 163.

FRANCESCHI, S., LA VECCHIA, C., HELMRICH, S.P., MANGIONI, C. & TOGNONI, G. (1982). Risk factors for epithelial ovarian cancer in Italy. *Am. J. Epidemiol.*, **115**, 714.

HARTGE, P., HOOVER, R., LESHER, L.P. & McGOWAN, L. (1983). Talc and ovarian cancer. *J. Am. Med. Assoc.*, **250**, 1844.

HILDRETH, N.G., KELSEY, J.L., LIVOLSI, V.A. & 5 others (1981). An epidemiologic study of epithelial carcinoma of the ovary. *Am. J. Epidemiol.*, **114**, 398.

JOLY, D.J., LILIENFELD, A.M., DIAMOND, E.L. & BROSS, I.D.J. (1974). An epidemiologic study of the relationship of reproductive experience to cancer of the ovary. *Am. J. Epidemiol.*, **99**, 190.

LESHER, L., McGOWAN, L., HARTGE, P. & HOOVER, R. (1985). Age at first birth and risk of epithelial ovarian cancer. *J. Natl Cancer Inst.*, **74**, 1361.

LONGO, D.L. & YOUNG, R.C. (1979). Cosmetic talc and ovarian cancer. *Lancet*, **ii**, 349.

McGOWAN, L., PARENT, L., LEDNAR, W & NORRIS, H.J. (1979). The woman at risk for developing ovarian cancer. *Gynecol. Oncol.*, **7**, 325.

MORI, M., KIYOSAWA, H. & MIYAKE, H. (1984). Case-control study of ovarian cancer in Japan. *Cancer*, **53,** 2746.

NASCA, P.C., GREENWALD, P., CHOROST, S., RICHART, R., & CAPUTO, T. (1984). An epidemiologic case-control study of ovarian cancer and reproductive factors. *Am. J. Epidemiol.*, **119,** 705.

NEIL, J.R., HAMMOND, G.T., NOBLE, A.D., RUSHTON, L. & LETCHWORTH, A.T. (1975). Late complications of sterilisation by laparoscopy and tubal ligation. *Lancet*, **ii,** 699.

NEWHOUSE, M.L., PEARSON, R.M., FULLERTON, J.M., BOESON, E.A.M. & SHANNON, H.S. (1977). A case control study of carcinoma of the ovary. *Br. J. Prev. Social Med.*, **31,** 148.

OFFICE OF POPULATION CENSUSES AND SURVEYS. (1970). *Classifications of Occupations*. HMSO: London.

RISCH, H.A., WEISS, N.S., LYON, J.L., DALING, J.R., & LIFF, J.M. (1983). Events of reproductive life and the incidence of epithelial ovarian cancer. *Am. J. Epidemiol.*, **117,** 128.

ROE, F.J.C. (1979). Controversy: cosmetic talc and ovarian cancer. *Lancet*, **ii,** 744.

TZONOU, A., DAY, N.E., TRICHOPOULOS, D. & 4 others (1984). The epidemiology of ovarian cancer in Greece: a case-control study. *Eur. J. Cancer Clin. Oncol.*, **20,** 1045.

WEISS, N.S., LYON, J.L., KRISHNAMURTHY, S., DIETERT, S.E., LIFF, J.M. & DALING, J.R. (1982). Noncontraceptive estrogen use and the occurance of ovarian cancer. *J. Natl Cancer Inst.*, **68,** 95.

WEST, R.O. (1966). Epidemiologic study of malignancies of the ovaries. *Cancer*, **19,** 1001.

WU, N.L., WHITTEMORE, A.S., PAFFENBARGER, R.S. & 7 others (1988). Personal and environmental characteristics related to epithelial ovarian cancer. 1. Reproductive and menstrual events and oral contraceptive use. *Am. J. Epidemiol.*, **128,** 1216.

WYNDER, E.L., DODO, H. & BARBER, H.R.K. (1969). Epidemiology of cancer of the ovary. *Cancer*, **23,** 352.

Exhibit 26

Perineal Exposure to Talc and Ovarian Cancer Risk

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***Objective*: We sought to determine whether the use of talc in genital hygiene increases the risk for epithelial ovarian cancer.**

***Methods*: We interviewed 235 white women diagnosed with epithelial ovarian cancer between 1984–1987 at ten Boston metropolitan area hospitals and 239 population-based controls of similar race, age, and residence.**

***Results*: Overall, 49% of cases and 39% of controls reported exposure to talc, via direct application to the perineum or to undergarments, sanitary napkins, or diaphragms, which yielded a 1.5 odds ratio (OR) for ovarian cancer (95% confidence interval [CI] 1.0–2.1). Among women with perineal exposure to talc, the risk was significantly elevated in the subgroups of women who applied it: 1) directly as a body powder (OR 1.7, 95% CI 1.1–2.7), 2) on a daily basis (OR 1.8, 95% CI 1.1–3.0), and 3) for more than 10 years (OR 1.6, 95% CI 1.0–2.7). The greatest ovarian cancer risk associated with perineal talc use was observed in the subgroup of women estimated to have made more than 10,000 applications during years when they were ovulating and had an intact genital tract (OR 2.8, 95% CI 1.4–5.4); however, this exposure was found in only 14% of the women with ovarian cancer.**

Conclusions*: These data support the concept that a lifetime pattern of perineal talc use may increase the risk for epithelial ovarian cancer but is unlikely to be the etiology for the majority of epithelial ovarian cancers. *(Obstet Gynecol 1992;80:19–26)

The theory that human ovarian cancer may be mesotheliomas that originate from asbestos exposure was first proposed by Graham and Graham.[1] Later, Parmley and Woodruff[2] suggested that effluences that may arise from the vagina, uterus, or tubes might enter the pelvic cavity and interact with ovarian surface epithelium to induce such mesotheliomas. Longo and Young[3] further speculated that cosmetic talc might act in this manner because of its chemical similarity to asbestos. Although there is little doubt that asbestos may induce mesotheliomas, the link between genital exposure to talc and ovarian cancer is less clear.

The few epidemiologic studies that have examined the association between talc and ovarian cancer reported only modest elevations of risk, but data on all potential sources of genital talc exposure were limited.[4–7] The purpose of this report was to present findings from a new case-control study of ovarian cancer conducted in the Boston metropolitan area, in which we considered a variety of modes for perineal talc exposure, the frequency and duration of use, and various reproductive characteristics that might influence the ability of talc to enter the pelvic cavity and affect the ovaries.

Materials and Methods

Between July 1984 and September 1987, we identified 394 women between 18–76 years of age diagnosed with borderline or malignant epithelial ovarian cancer at one of ten participating hospitals in the Boston metropolitan area. Permission to contact each patient was obtained in advance from the physician of record. An in-person interview was conducted with 272 (69%) of the 394 cases identified. Thirty-one percent were not interviewed because of physician and/or patient refusal, patient death, or relocation. The final sample for analysis was further restricted to the 235 white women confirmed as having an epithelial ovarian tumor based on an independent pathology review conducted by two of the authors (DAB and WRW).

Controls were selected from the Massachusetts Town Books, annual publications that list residents by name, age, and address according to voter precincts. For each new ovarian cancer case interviewed, a ran-

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Table 1. Influence of Any Perineal Talc Exposure* on Ovarian Cancer Risk by Characteristics of Study Participants, Boston Metropolitan Area, 1984–1987

	Cases		Controls			
	Total	Talc exposure	Total	Talc exposure	Crude OR	95% CI
All subjects	235	114 (48.5%)	239	94 (39.3%)	1.5	1.0–2.1
Age (y)						
<50	96	41 (42.7%)	101	28 (28.0%)	1.9	1.2–3.4
≥50	139	73 (52.5%)	138	66 (47.8%)	1.2	0.8–2.1
Education (y)						
≤12	93	51 (54.8%)	115	48 (41.7%)	1.7	1.1–3.0
>12	142	63 (44.4%)	124	46 (37.1%)	1.4	0.9–2.4
Marital status						
Never married	40	14 (35.0%)	24	6 (25.0%)	1.6	0.5–5.7
Ever married	195	100 (51.3%)	215	88 (40.9%)	1.5	1.0–2.7
Religion						
Jewish	35	21 (60.0%)	21	12 (57.1%)	1.1	0.4–3.9
Non-Jewish	200	93 (46.5%)	218	82 (37.6%)	1.4	0.9–2.5
Weight (lb)						
<140	123	53 (43.1%)	125	49 (39.2%)	1.2	0.7–1.9
≥140	112	61 (54.5%)	114	45 (39.2%)	1.8	1.1–3.1
Use of OCs (mo)						
≥3	66	31 (47.0%)	82	27 (32.9%)	1.8	0.8–4.5
<3 or never	169	83 (49.1%)	157	67 (42.7%)	1.3	0.8–2.3
No. of live-born children						
0	79	30 (38.0%)	43	12 (27.9%)	1.6	0.7–4.0
1	31	21 (67.7%)	27	5 (18.5%)	9.2	2.9–46.2
2	40	24 (60.0%)	63	26 (41.3%)	2.1	0.9–3.3
≥3	85	39 (45.9%)	106	51 (48.1%)	0.9	0.6–1.6

OR = odds ratio; CI = confidence interval; OCs = oral contraceptives.
* Sources of perineal talc exposure include: dusting of underwear, diaphragms, or sanitary napkins; use by partner on his perineal area; use as a body powder.

dom number generator selected one page from the town book corresponding to the case's precinct of residence. By working forward in the town book, we selected the first five female subjects within 2 years of age of the case as potential controls. Of these five, the first subject of the same race as the case without a history of a bilateral oophorectomy was asked to participate in the study. Of the 526 controls contacted, 239 interviews were conducted (25% could not be reached, 10% reported a history of bilateral oophorectomy, and 19% declined to participate). Further details of the study methods can be found elsewhere.[8]

The in-person interview focused on the following: demographic and occupational history; medical and reproductive histories, including pregnancies, hormones used, and gynecologic operations; dietary history; cigarette smoking; and hygienic practices. The hygienic practices included information regarding the use of douches, type of sanitary protection used, and perineal exposure to talc. Queried sources of perineal talc exposure included dusting of underwear, sanitary napkins, and diaphragms; exposure via husband's use of talc; and more direct exposure to the perineum as a body powder. No reliable information on talc exposure during infancy with diapering could be obtained, and women using talc as a body powder on areas other than the perineum were considered nonexposed. For each exposure, we inquired about brands used, age at first use, total years of use, and frequency of use per month, to enable us to estimate the total lifetime number of applications from all sources of exposure.

Differences between cases and controls in the distribution of these various exposures to talc were examined both qualitatively and quantitatively. The influence of confounders and effect modifiers was assessed first through stratification and then using unconditional logistic regression.[9] The primary matching variable, age, was retained in each logistic model. The χ^2 test for linear trend was calculated based on the change in deviance in models with and without continuous exposure variables.[9]

Results

Table 1 shows the proportion of cases and controls with any reported perineal talc exposure, and the associated crude exposure odds ratio (OR), by certain demographic and reproductive subgroups. Overall, a

Table 2. History of Talc Exposure by Types of Application, Brand of Powders, Years and Frequency of Use, and Era of Use

	Cases	Controls	Adjusted OR*	95% CI
No genital talc application	121 (51.5%)	145 (60.7%)	1.0	
Any genital talc application	114 (48.5%)	94 (39.3%)	1.5	1.0–2.1
Type of application				
Only via sanitary napkins and/or underwear	9 (3.8%)	12 (5.0%)	1.1	0.4–2.8
Via partner or applications to diaphragm†	20 (8.5%)	21 (8.8%)	1.2	0.6–2.4
Via dusting powder to perineum†	85 (36.2%)	61 (25.5%)	1.7	1.1–2.7
Applications of talc per month				
<5	32 (13.6%)	28 (11.7%)	1.5	0.8–2.7
5–29	24 (10.2%)	25 (10.5%)	1.2	0.6–2.2
≥30	58 (24.7%)	41 (16.7%)	1.8	1.1–3.0
Years of talc use				
<10	14 (6.0%)	15 (6.3%)	1.2	0.5–2.6
10–29	49 (20.9%)	39 (16.3%)	1.6	1.0–2.7
≥30	51 (21.7%)	40 (16.3%)	1.6	1.0–2.7
Age (y) at first talc use				
<20	66 (28.1%)	50 (20.9%)	1.7	1.1–2.7
20–25	27 (11.5%)	26 (10.9%)	1.2	0.6–2.2
>25	21 (8.9%)	18 (7.5%)	1.6	0.8–3.2
Years since last talc use				
Within last 6 mo	48 (20.4%)	27 (11.3%)	2.3	1.3–4.0
Between 6 mo–10 y	36 (15.3%)	39 (16.3%)	1.1	0.7–1.9
10 y or more	30 (12.8%)	28 (11.7%)	1.4	0.8–2.6
Era of use‡				
Exclusive use after 1960	29 (12.3%)	30 (12.6%)	1.1	0.6–2.1
Any use before 1960	75 (31.9%)	57 (23.9%)	1.7	1.1–2.7
Brand of application§				
Brand or generic baby powder	91 (38.7%)	72 (30.1%)	1.6	1.1–2.5
Deodorizing or other scented powders	16 (6.8%)	17 (7.2%)	1.2	0.6–2.5

OR = odds ratio; CI = confidence interval.

* Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), use of sanitary napkins (no, yes), douching (no, yes), age (continuous), and weight (<140 lb, ≥140 lb).

† Includes combinations with sanitary napkins or underwear.

‡ Restricted to women older than 10 years in 1960; 100 cases and 118 controls were unexposed and used as the referent group.

§ Excludes seven cases and five controls with unknown powders. Seven cases and four controls reported combinations of more than one brand and were classified according to the brand used most frequently and for the longest period. Specific brands mentioned by cases were: Johnson and Johnson, 71; generic baby powder, 20; Shower to Shower, four; other scented, 12. Specific brands mentioned by controls were: Johnson and Johnson, 54; generic baby powder, 18; Shower to Shower, three; other scented powder, 14.

greater percentage of cases (48.5%) than controls (39.3%) reported any perineal exposure to talc-containing powders. Subgroups of controls in which exposure to talc appeared to be more common were women older than 50 years ($P = .002$), ever married ($P = .12$), Jewish ($P = .08$), and parous ($P = .05$). Controls who reported no oral contraceptive (OC) use also reported greater talc use. However, this interaction may be explained by the older age distribution among controls who reported perineal application of talc. We observed stronger associations between talc and ovarian cancer risk in the subgroups of cases and controls younger than age 50, less well educated, heavier than 140 lb 5 years before diagnosis, and reporting a history of one or two live births. The number of live births was the only factor that produced statistically significant heterogeneity in the ORs for the talc and ovarian cancer association. Age, education, marital status, religion, weight, and parity were considered confounders and were included as covariates in subsequent multivariate models. Use of OCs did not confound the talc-ovarian cancer association.

Table 2 examines the association between talc use and ovarian cancer by variables related to the specific types and kinds of applications, and measures of duration including length, frequency, and period of use. Compared with women with no genital talc exposure, women exposed to talc only through use as a dusting powder on sanitary napkins or underwear had no appreciable increased risk for ovarian cancer. There was also no substantial increase in risk among women exposed to talc only through a husband's use or use in the storage of diaphragms, or in combination with applications to sanitary napkins or underwear. The most frequent method of perineal talc exposure was use as a dusting powder directly to the perineum,

Table 3. Estimated Total Lifetime Perineal Applications of Talc-Containing Powders in Cases and Controls

Applications	Cases	Controls	Adjusted OR*	95% CI
Total applications				
None	121	145	1.0	
<1000	18	19	1.3	0.7–2.7
1000–10,000	54	44	1.5	0.9–2.4
>10,000	42	31	1.8	1.0–3.0
χ^2 1*df* test for linear trend = 2.85, $P = .094$†				
Applications excluding use after hysterectomy or tubal ligation				
None	121	145	1.0	
<1000	19	19	1.4	0.7–2.9
1000–10,000	57	46	1.5	0.9–2.4
>10,000	38	29	1.7	1.0–3.0
χ^2 1*df* test for linear trend = 3.19, $P = .077$†				
Applications excluding use after hysterectomy or tubal ligation, and use during nonovulatory months‡				
None	124	149	1.0	
<1000	24	23	1.5	0.8–2.9
1000–10,000	55	51	1.3	0.8–2.0
>10,000	32	16	2.8	1.4–5.4
χ^2 1*df* test for linear trend = 6.15, $P = .015$†				

Abbreviations as in Table 2.

* Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), use of sanitary napkins (no, yes), douching (no, yes), age (continuous), and weight (<140 lb, ≥140 lb).

† Trend test based on actual applications as a continuous variable.

‡ Excludes exposures while taking oral contraceptives, while pregnant or breast-feeding, or occurring after menopause. There were three cases and four controls who moved from "exposed" to "nonexposed" in this category, as all of the exposure occurred during oral contraceptive use, pregnancies, or after menopause.

alone or in combination with either a partner's use or use in the storage of diaphragms. This exposure occurred in 85 cases (36.2%) and 61 controls (25.5%) ($P = .01$). Of the application modes studied, direct perineal application produced the greatest risk (OR 1.7, 95% confidence interval [CI] 1.1–2.7).

We also examined the talc-ovarian cancer association by frequency and years of use (Table 2). When monthly frequency was considered as a continuous variable in the logistic model, the χ^2 linear test of trend was 4.06 ($P = .046$), indicating that the risk for ovarian cancer increased significantly with increasing frequency of applications per month. The categorical analysis showed that relative to non-users, the risk was greatest in women who applied talc at least once per day. When years of use was included as a continuous variable, the test for linear trend was 3.32 ($P = .07$). The categorical analysis showed that relative to non-users, women who applied talc for more than 10 years were at 60% greater risk for ovarian cancer. Likewise, perineal applications of talc early in life (before age 20) or applications within 6 months of diagnosis (reference age for controls) produced the stronger ORs.

To assess whether the risk of ovarian cancer with perineal exposure to talc was affected by the time when talc-containing products were manufactured, we examined the association separately in women who only used talcum powder after 1960 and in women who reported any use of talcum powder before 1960 (Table 2). After restricting the population to women older than age 10 in 1960 and adjusting for age, parity, and a number of other demographic characteristics, we found that the association of talc and ovarian cancer was greater in women using talc products before 1960 ($P = .025$).

The last entry in Table 2 shows the risks by brand of powder used. No subjects could recall exclusive use of starch-based powders. Brand or generic "baby powder" was used most frequently and was the category associated with a statistically significant risk for ovarian cancer. With respect to other powders, four cases and three controls reported primary use of deodorizing powders, and 12 cases and 14 controls reported primary use of other scented powders. It should be appreciated that, because the period of exposure often occurred over decades, verification of brands was not possible.

Table 3 examines the ovarian cancer risk associated with the total number of applications to the perineum, estimated by cumulating frequency and years of use for the various kinds of exposures. An 80% excess risk was associated with an estimated exposure of more than 10,000 applications (equivalent to daily use for 30

Table 4. Adjusted Odds Ratios and 95% Confidence Intervals for Ovarian Cancer by Any Perineal Exposure to Talc* and Indicators of Ovulation and Tubal Occlusion

	Cases		Controls			
	Total	Talc exposure	Total	Talc exposure	Adjusted OR†	95% CI
All subjects	235	114 (48.5%)	239	94 (39.3%)	1.5	1.0–2.1
Mid-cycle pain						
No	181	88 (48.6%)	184	77 (41.9%)	1.4	0.9–2.2
Yes	54	26 (48.2%)	55	17 (30.9%)	2.0	0.8–5.2
Regular period						
No	26	12 (46.2%)	34	16 (47.1%)	1.1	0.4–3.4
Yes	209	102 (48.8%)	205	78 (38.1%)	1.7	1.1–2.5
PID or ectopic pregnancy						
No	226	113 (50.0%)	230	92 (40.0%)	1.6	1.1–2.4
Yes	9	1 (11.1%)	9	2 (22.2%)	0.1	0.01–7.0

PID = pelvic inflammatory disease; other abbreviations as in Table 2.
* Sources of perineal talc exposure include: dusting of underwear, diaphragms, or sanitary napkins; use by partner on his perineal area; use as a body powder.
† Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), use of sanitary napkins (no, yes), douching (no, yes), age (continuous), and weight (<140 lb, ≥140 lb).

years) as compared with non-users. When considered as a continuous variable in the logistic model, the χ^2 linear test of trend was 2.85 ($P = .094$). The remaining entries in Table 3 show how conditions that either close the upper genital tract or are associated with anovulation affect the dose response of number of applications on ovarian cancer risk. The second entry shows the effect of censoring applications that occurred after tubal ligation or hysterectomy. No appreciable change in the ORs or the dose response was noted. The third entry shows the effect of censoring applications after hysterectomy and tubal ligation and use during presumed nonovulatory periods. Excluded were exposures occurring while taking OCs, while pregnant or breast-feeding, and after menopause. The risk associated with fewer than 10,000 applications was not substantially altered. However, the risk associated with more than 10,000 applications was nearly threefold and statistically significant. The χ^2 test for a linear trend of risk, by number of applications as a continuous variable, increased to 6.15 ($P = .015$).

Table 4 shows the association of talc exposure and ovarian cancer based on other clinical factors that may predict either ovulation or tubal occlusion. The ORs were greater in women with a history of mid-cycle pain or regular periods—potential clinical predictors of ovulatory cycles. An association between talc and ovarian cancer was absent in women with a history of either pelvic inflammatory disease or ectopic pregnancy—potential markers of a closed genital tract. However, only nine cases and nine controls reported a history of pelvic inflammatory disease or ectopic pregnancy, and the CI on the exposure OR was correspondingly wide.

Table 5 shows the relative risk for any perineal use of talc when restricted to specific histologic type and grade of ovarian tumors. The greatest association was found in women diagnosed with an endometrioid tumor or borderline ovarian tumor.

Discussion

Animal and epidemiologic studies have addressed the plausibility of an association between talc and ovarian cancer. Intraperitoneal injection of talc in rodents produced papillary changes in the surface epithelium not inconsistent with the first stage in the development of surface papillary epithelial neoplasms.[10] However, because the ovaries of small rodents are surrounded by a

Table 5. History of Talc Use by Histologic Type and Grade

Histologic type	Any use of talc	No use of talc	Adjusted OR*	95% CI
Controls	94	145	1.0	
Histologic type				
Serous	60	64	1.4	0.9–2.2
Mucinous	17	25	1.2	0.6–2.5
Endometrioid	18	11	2.8	1.2–6.4
Other	19	21	1.6	0.8–3.3
Histologic grade				
Borderline	32	30	2.4	1.2–4.5
Grade 1	6	11	1.0	0.3–2.8
Grade 2	21	22	1.5	0.7–3.0
Grade 3	27	24	1.5	0.8–2.8
Undifferentiated	28	34	1.2	0.7–2.2

Abbreviations as in Table 2.
* Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), age (continuous), and weight (<140 lb, ≥140 lb).

Table 6. Odds Ratios With 95% Confidence Intervals of Ovarian Cancer in Relation to Any Perineal Exposure to Talc as Reported in Previous Epidemiologic Studies

Author(s) (year)	Cases		Controls		Crude OR	95% CI
	Total	Talc exposure	Total	Talc exposure		
Cramer et al[4] (1982)	215	92 (42.8%)	215	61 (28.4%)	1.9	1.3–2.9
Hartge et al (1983)*	135	67 (49.6%)	171	100 (58.5%)	0.7	0.4–1.1
Whittemore et al[5] (1988)	188	98 (52.1%)	539	248 (46.0%)	1.4	0.9–2.0
Harlow and Weiss[6] (1989)†	116	49 (42.2%)	158	64 (40.5%)	1.1	0.7–2.1
Booth et al[7] (1989)	217	141 (65.0%)	434	256 (59.0%)	1.3	0.9–1.9
Harlow et al (1992) (current study)	235	114 (48.5%)	239	94 (39.3%)	1.5	0.9–1.8
All studies‡	1106	561 (50.7%)	1756	823 (46.9%)	1.3	1.1–1.6

Abbreviations as in Table 2.

* Hartge P, Hoover R, Lesher LP, et al. Talc and ovarian cancer (letter). JAMA 1983;250:1844. Odds ratio may include nonperineal talc exposure as well.

† Restricted to borderline ovarian tumors.

‡ Meta-analysis.[19]

peritoneal bursa which often becomes occluded and distended with follicular fluid after intraperitoneal injection of foreign bodies,[1] it is difficult to distinguish whether the papillary changes are the effects of foreign-body exposure or bursal distention. It would be worthwhile to repeat the talc experiments in guinea pigs or rabbits, the animals used in the original research of Graham and Graham.[1]

Is there evidence to suggest that talc can translocate from the vagina to the peritoneal cavity? It is known that red cells and endometrial tissue are capable of retrograde flow from the fallopian tubes.[11] Experiments in rats confirmed the presence of talc in the ovaries after the introduction of a talc suspension into the vagina and cervical os.[12] In humans, two studies observed the migration of inert carbon particles[13] and radioactively labeled human albumin microspheres[14] from the vagina to the fallopian tubes. In addition, several investigators have observed birefringent crystals embedded in ovarian tissue.[15–17] However, critics have argued that these findings resulted from poorly designed studies or lack of precision in measuring particulates, due to leaching of radionucleotide markers from the test materials or the introduction of contaminants during tissue processing.[18] In six multiparous cynomolgus monkeys separately caged, no translocation of talc was observed after 30 consecutive days of douching with a suspension of neutron-activated talc coupled with weekly injections of oxytocin.[18] However, this study was not able to address the effects of long-term use or coitus, which might facilitate talc translocation.

Several epidemiologic studies[4–7] have addressed the genital talc-ovarian cancer association (Table 6). Each study has consistently reported little or no association with the use of talc-dusted diaphragms, and stronger associations with direct perineal application. Dose response of perineal talc exposure from all sources by frequency or years of use was not available in the studies by Hartge et al (Hartge P, Hoover R, Lesher LP, et al. Talc and ovarian cancer [letter]. JAMA 1983;250:1844), Harlow and Weiss,[6] or Cramer et al.[4] Whittemore et al[5] reported no significant dose response by years or frequency of use, whereas Booth et al[7] reported a marginally significant trend with frequency of use. Using the techniques of meta-analysis, in which ORs from multiple studies are weighted by their variances,[19] we calculated a statistically significant OR of 1.3 for any perineal talc exposure and ovarian cancer risk (95% CI 1.1–1.6) from the various studies. We therefore conclude that there is an association, albeit modest, between ovarian cancer and perineal talc use. However, insufficient detail has been available to rule out a stronger association in certain subgroups of users.

Our study included greater detail on genital talc use, including methods, frequency, and years of use (Table 2). Talc applied as a dusting powder directly to the perineum carried a greater risk than less direct exposure via a partner's use or the dusting of undergarments, sanitary napkins, or diaphragms. Current use of talc was associated with a greater ovarian cancer risk than past use. Daily versus less than daily talc use, and talc use for more than 10 years versus less than 10 years, were associated with greater risk for ovarian cancer.

Most subjects reported use of "baby powder." We were unable to confirm a previous finding that powders with "deodorizing" agents were associated with particular risk.[6] Thus, this study failed to answer a key issue in the talc-ovarian cancer association: whether the risk pertains to all cosmetic talcs or only to certain

preparations likely to be contaminated by asbestos. Cralley et al[20] and Rohl et al[21] found considerable variation in the purity of cosmetic talcs, with fiberform contents varying from less than 1 to 30%. Most of these products were manufactured before 1970 and it is likely that the asbestiform content has decreased since 1976, when manufacturers instituted voluntary guidelines on asbestos contamination. Our finding of a lower ovarian cancer risk in women with exclusive use of talc after 1960 may support this theory. Because of the difficulty in obtaining a complete and detailed history of powders used, the issue of whether risk pertains only to asbestos-contaminated powders may need to be settled by animal experiments.

In our analysis, we first calculated all genital applications of talc based upon frequency and years of use. As a continuous variable in a multivariate model, no significant dose response was observed between total genital applications of talc and ovarian cancer risk. Because the "translocation" theory assumes an open genital tract, we then excluded application after tubal ligation or hysterectomy, but observed no appreciable change in the dose response. Further restricting talc exposure to months when the women were likely to be ovulatory, we observed a significant dose response, such that women with an intact genital tract and more than 10,000 applications during ovulatory cycles had nearly a threefold increase in risk for ovarian cancer. Some additional evidence that might support an interaction with ovulation includes stronger associations in women with regular periods and mid-cycle pain. Cramer et al[4] suggested that talc contamination around the time of ovulation might lead to the incorporation of talc particulates into inclusion cysts that may form with ovulation. Experiments on foreign-body tumorigenesis have shown that implantation of foreign bodies into the lumens of epithelial-lined organs provides a favorable environment for carcinogenesis.[22] Alternatively, Mostafa et al[16] speculated that foreign-body exposure might produce cortical "granulomas" whose link to stromal hyperactivity (and hormonally related cancers) is argued in older literature.[23]

We observed that the talc association was strongest in women with endometrioid or borderline ovarian tumors. However, an earlier study by Cramer et al[4] reported no such variation in risk by histologic subtype. It was noted that a greater proportion of women with endometrioid tumors than with other histologic types of ovarian cancer reported more than 10,000 lifetime applications of talc during ovulatory cycles while having an intact genital tract (34 versus 16%). Although this may explain in part the strong talc-ovarian cancer association noted in women with endometrioid tumors, it does not explain the strong association noted in women with borderline ovarian tumors, of whom only 13% reported long-term talc exposure. This variation in risk among histologic subtypes may reflect a chance finding or a need to examine endometrioid and borderline tumors more carefully for evidence of a foreign-body effect.

An unusual observation was the strong association between talc use and ovarian cancer in the subgroup of women with one or two pregnancies but a lower risk in women with either no children or three or more children. Although chance may be the most likely explanation for this finding, we wonder whether this peculiar interaction might reflect, in part, an effect of pregnancy on the degree of openness of the cervical os and its ability to allow translocation of vaginal particulates. The parous cervix has a larger os than the nulliparous cervix, and this could explain the greater risk in women with one child compared with women with no live births. At the other extreme, multiple pregnancies may offer other protective mechanisms (such as reduced ovulation as discussed above) that could outweigh this effect. Clearly, this is a very speculative hypothesis but one that might be tested in experimental studies (ie, repeating the vaginal talc experiments in nulliparous and parous mice or primates).

Noncausal explanations are possible in any epidemiologic research. We cannot rule out the possibility of differential over- or under-reporting of talc exposure in our cases and controls, especially in those with reproductive events that enhance ORs. In addition, though we were successful in interviewing 69% of eligible ovarian cancer cases and 81% of eligible controls contacted, we cannot assess whether the cases and controls not interviewed could have selectively differed in their reproductive characteristics or in their use of talc-containing powders. Because our associations are based upon responses from participating cases and controls, the validity of our results depends upon the assumption that respondents and non-respondents were similar with respect to talc and other relevant exposures, or that the magnitude of any respondent-non-respondent difference was similar for cases and controls. Because the interview provided the only source of "exposure" information, we were unable to assess the likelihood of this assumption. The extent of this bias, however, is likely to be small because the reproductive characteristics and history of talc exposure in participating cases and controls are, for the most part, reasonably consistent with earlier epidemiologic studies of ovarian cancer. Finally, in our attempt to present the most accurate ORs, we made a variety of adjustments to account for the confounding influence of factors associated with both ovarian cancer risk and

talc exposure. Nevertheless, we cannot rule out the presence of other unknown factors that might have influenced, in part, our observed associations.

Because the overall association between genital use of talc and ovarian cancer remains weak, it is unlikely that this exposure-disease pathway is the principal one involved in ovarian cancer etiology. We have previously discussed the role of dietary and metabolic factors in a model for ovarian cancer involving gonadotropin stimulation of failing ovaries.[8] Even if an etiologic association were to pertain in the subgroups of daily users or users with more than 10,000 applications during ovulatory months, we calculate that by applying these ORs to the exposure rate among cases,[24] the proportion of ovarian cancer incidence attributable to this level of talc exposure is about 10%. Nevertheless, given the poor prognosis for ovarian cancer, any potentially harmful exposures should be avoided, particularly those with limited benefits. For this reason, we discourage the use of talc in genital hygiene, particularly as a daily habit.

References

1. Graham J, Graham R. Ovarian cancer and asbestos. Environ Res 1967;1:115–28.
2. Parmley TH, Woodruff JD. The ovarian mesothelioma. Am J Obstet Gynecol 1974;120:234–41.
3. Longo DL, Young RC. Cosmetic talc and ovarian cancer. Lancet 1979;ii:349–51.
4. Cramer DW, Welch WR, Scully RE, Wojciechowski CA. Ovarian cancer and talc. Cancer 1982;50:372–6.
5. Whittemore AS, Wu ML, Paffenbarger RS, et al. Personal and environmental characteristics related to epithelial ovarian cancer. II. Exposures to talcum powder, tobacco, alcohol, and coffee. Am J Epidemiol 1988;128:1228–40.
6. Harlow BL, Weiss NS. A case-control study of borderline ovarian tumors: The influence of perineal exposure to talc. Am J Epidemiol 1989;130:390–4.
7. Booth M, Beral V, Smith P. Risk factors for ovarian cancer: A case-control study. Br J Cancer 1989;60:592–8.
8. Cramer DW, Harlow BL, Willett WC, et al. Galactose consumption and metabolism in relation to the risk of ovarian cancer. Lancet 1989;ii:66–71.
9. Breslow NE, Day NE. Statistical methods in cancer research. Vol 1. The analysis of case control studies. IARC scientific publication no 32. Lyon: International Agency for Research on Cancer, 1980.
10. Hamilton TC, Fox H, Buckley CH, Henderson WJ, Griffiths K. Effects of talc on the rat ovary. Br J Exp Pathol 1984;65:101–6.
11. Sampson JA. The development of the implantation theory for the origin of endometriosis. Am J Obstet Gynecol 1940;40:549–57.
12. Henderson WJ, Hamilton TC, Baylis MS, et al. The demonstration of the migration of talc from the vagina and posterior uterus to the ovary in the rat. Environ Res 1986;40:247–50.
13. Egli GE, Newton MD. The transport of carbon particles in the human female reproductive tract. Fertil Steril 1961;12:151–5.
14. Venter PF, Iturralde M. Migration of particulate radioactive tracer from the vagina to the peritoneal cavity and ovaries. S Afr Med J 1979;55:917–9.
15. Henderson WJ, Joslin CAF, Turnbull AC, Griffiths K. Talc and carcinoma of the ovary and cervix. J Obstet Gynaecol Br Commonw 1971;78:266–72.
16. Mostafa SAM, Bargeron CB, Flower RW, Rosenshein NB, Parmley TH, Woodruff JD. Foreign body granulomas in normal ovaries. Obstet Gynecol 1985;66:701–2.
17. Griffiths K, Henderson WJ, Chandler JA, Joslin CAF. Ovarian cancer: Some new analytical approaches. Postgrad Med J 1973;49: 69–72.
18. Wehner AP, Hall AS, Weller RE, Lepel EA, Schirmer RE. Do particles translocate from the vagina to the oviducts and beyond? Food Chem Toxicol 1985;23:367–72.
19. Greenland S. Quantitative methods in the review of epidemiologic literature. Epidemiol Rev 1987;9:1–30.
20. Cralley LJ, Key MM, Groth DH, Lainhart WS, Ligo RM. Fibrous and mineral content of cosmetic talcum products. Am Ind Hyg Assoc J 1968;29:350–4.
21. Rohl AN, Langer AM, Selikoff IJ, et al. Consumer talcums and powders: Mineral and chemical characterization. J Toxicol Environ Health 1976;2:255–84.
22. Brand KG, Johnson KH, Buoen LC. Foreign body tumorigenesis. Crit Rev Toxicol 1976;4:353–94.
23. Woll E, Hertig AT, Smith GVS, et al. The ovary in endometrial carcinoma with notes on the morphological history of the aging ovary. Am J Obstet Gynecol 1948;56:617–33.
24. Rothman KJ. Modern epidemiology. Boston: Little, Brown, 1986: 35–40.

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Exhibit 27

Occupation and Ovarian Cancer: A Case-Control Study in the Washington, DC, Metropolitan Area, 1978–1981

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Ovarian cancer risk factors may be genetic, reproductive, or hormonal in nature. Occupational exposure to talc and other carcinogenic substances has not been studied in relation to ovarian cancer risk. We therefore examined the job histories of 296 women aged 20 to 79 who were diagnosed with epithelial ovarian cancer in the Washington, DC area in 1978 to 1981, comparing them to 343 hospital controls, matched for age and race. A blind exposure assessment, evaluating each job/industry combination for potential exposure to talc, ionizing radiation, polycyclic aromatic hydrocarbons, and solvents was conducted by an industrial hygienist blind to case-control status. Women exposed to talc had a relative risk of ovarian cancer below the null, but the confidence interval was wide and there was no evidence of a trend. Women exposed to polycyclic aromatic hydrocarbons had an elevated relative risk, also with a wide confidence interval and no evidence of a trend with duration.

Each year, more than 20,000 women in the United States develop ovarian cancer, and more than 12,000 die from it.[1] Risk of developing ovarian cancer (either invasive or of low malignant potential) is negatively correlated with number of pregnancies, years of use of oral contraceptives, hysterectomy despite ovarian preservation, and, to a lesser extent, years of breastfeeding.[2,3,4] Risk is positively associated with a family history of ovarian or breast cancer and with a history of infertility. Use of talcum powder has been suggested but not confirmed as a modest risk factor.[5,6]

No occupational risk factors have been established, but few studies have examined the issue. As with most case-control studies, many individual job titles are reported by so few women that it is hard to assess risk by occupation.[7] Similarly, in many occupational cohorts, so few ovarian cancers are expected that it also would be hard to detect moderately elevated risks.

We reviewed the occupational histories in a study of ovarian cancer cases and hospital controls for two purposes. We wished to determine which jobs were reported by enough women to permit an occupational analysis in this or similar studies of women in the cancer age range. Second, examination of job histories by an industrial hygienist may reveal associations of disease with certain occupational exposures, associations that cannot be discerned by looking only at types of industries or job titles.[8] Industries tend to have hetero-

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geneous exposures that are often dependent on the job, and jobs tend to have heterogeneous exposures that are often dependent on the industry. In addition, in most case-control studies, the number of people holding any particular job is small. Even if an elevated risk is found, it is likely to have large confidence intervals, thus making the association suspect.

Having no strong hypotheses, we decided to examine occupational exposure to talc, because use of cosmetic talc has been suggested as a risk factor. We also looked at several exposures related to other malignancies or that could have been experienced in jobs held by the study subjects (eg, ionizing radiation, polycyclic aromatic hydrocarbons (PAHs),[9] and solvents).[10]

Methods

Details of this study have been reported previously.[7] Cases were 296 women diagnosed with epithelial ovarian cancer during 1978–1981 in hospitals in the Washington, DC metropolitan area. Controls were 343 women discharged from the same hospitals for conditions unrelated to any of the exposures under study and matched for age and race. All ovarian cancer cases were confirmed by pathology review. Study subjects were interviewed by trained interviewers using a standardized questionnaire that took approximately 1 hour to complete. The questionnaire included lifetime job history and a specific question about exposure to talc on the job. Each industry and occupation reported was assigned a Bureau of the Census[11] code by trained coders.

One of the authors (PS) conducted an industrial hygiene exposure assessment, evaluating each job/industry combination for potential exposure to talc, ionizing radiation, PAHs, and solvents blind to case-control status. Probability of exposure was assigned on a scale of 0 to 4 (definitely not, unlikely, possibly, probably, definitely), and where probability exceeded 0, level of exposure was assigned on a scale of 1 to 3 (low, medium, high). Evaluation of jobs was based on the industrial hygienist's experience.

We estimated relative risk accord-

TABLE 1
Number of Ovarian Cancer Cases and Controls Ever Employed in the 20 Job Titles Reported Most Frequently

Job Title	Cases (n = 296)	Controls (n = 343)
Secretary	80	92
Typist	36	34
Salesperson, sales clerk	34	32
Bookkeeper	27	21
Clerical worker (not otherwise specified)	28	34
Registered nurse	9	17
Waitress	12	21
Manager or administrator	18	14
Stenographer	14	10
Public administration official	18	10
Teacher (excluding elementary and secondary)	11	13
Miscellaneous clerical worker	12	16
Editor or reporter	12	7
Elementary school teacher	15	8
Cashier	10	11
Secondary school teacher	11	11
Receptionist	12	10
Telephone operator	9	11
Cleaner	7	9
Office manager	6	10

TABLE 2
Percentage Distribution of Selected Risk Factors for Cases and Controls Combined Who Worked at Least 5 Years in Four Common Occupations

	Secretary* (n = 205)	Teacher† (n = 49)	Nurse‡ (n = 28)	Cleaner§ (n = 22)
Livebirths				
0	28%	26%	21%	32%
1–2	43%	59%	32%	28%
3+	29%	14%	46%	46%
Oral contraceptives				
Never	79%	69%	79%	82%
Ever	20%	31%	21%	14%
Gynecologic surgery				
No	69%	75%	54%	59%
Yes	31%	25%	46%	41%
Infertility				
No	78%	78%	86%	91%
Yes	22%	22%	14%	9%
Menopausal estrogen use				
Never	67%	84%	75%	77%
Ever	33%	16%	25%	23%
Cigarette use				
Never	42%	57%	39%	73%
Ever	58%	43%	61%	27%

* Secretary occupation includes codes 303 (billing clerks), 325 (file clerks), 341–355 (office machine operators), 360 (payroll clerks), 370–372 (secretaries), 375 (statistical clerks), 376 (stenographers), 391 (typists), 394 (miscellaneous clerical workers), and 395 (not specified clerical workers).

† Teacher occupation includes codes 102–145 (teachers) and 382 (teacher aides).

‡ Nurse includes codes 075 (registered nurses), 925 (aides), and 926 (practical nurses).

§ Cleaner includes codes for private household cleaning: 982 (private household housekeepers), 902 (cleaners), 903 (janitors), 901 (maids), 950 (housekeepers), 983 (laundresses, private household), 984 (maids, private household), and other types of cleaning jobs.

ing to exposure with adjustment for confounders by fitting a logistic regression model.[12] The variables included race, age (in decade), parity (0, 1–2, 3 or more), gynecologic surgery (four categories), and duration of employment (none, less than 5 years, 5 to 9 years, 10 or more years) in jobs with the exposure of interest, using a probability rating of 2 to 4.

Results

A few job titles were reported very frequently, including secretarial and clerical, teaching, and nursing (Table 1). Private household cleaning was mentioned by only 16 subjects, but other types of cleaning jobs were reported, which we combined for our analysis. These common job titles were reported about as frequently by cases and controls.

For four common occupations, we combined job titles (eg, elementary, secondary, and other teachers). We compared women who had worked for at least 5 years in the occupation to women who never had, adjusting for age, race, parity, and gynecologic surgery. Secretaries and clerks had a relative risk (RR) of 1.1 (95% confidence interval [CI] = 0.7 to 1.8). For teachers, nurses, and cleaners, the RRs and CIs were 1.4 (0.8 to 3.4), 0.5 (0.2 to 1.0), and 0.7 (0.2 to 2.8), respectively.

We also assessed the relation between these four common occupations and major risk factors for ovarian cancer, as well as cigarette smoking, which is probably not related to risk (Table 2). This analysis was restricted to women who had worked in the occupation for at least 5 years. Parity was highest among nurses and cleaners and lowest among teachers. Oral contraceptive use was highest in teachers and lowest in cleaners. Nurses reported more gynecologic surgery and less cigarette smoking than did women in the other jobs. Controlling for the effect of these exposures did not alter the RR estimates for these common occupations.

The relation between the selected exposures and RR is shown in Table 3. Women exposed to talc had risks below the null, but the risks were not statistically significant. In the group with 5 to 9 years of exposure to PAHs, risks of ovarian cancer were elevated, but were not statistically significant. No other risks were elevated.

TABLE 3
Estimated Relative Risk of Ovarian Cancer, According to Length of Occupational Exposure to Solvents, Talc, and Polycyclic Aromatic Hydrocarbons*

Exposure†	Cases	Controls	Relative Risk	95% Confidence Interval
Solvents				
None	204	231	1.0	Referent
<5 years	32	31	1.1	0.7–2.0
5–9 years	12	19	0.7	0.3–1.6
10+ years	27	35	0.9	0.5–1.6
Talc				
None	263	285	1.0	Referent
<5 years	5	11	0.5	0.1–1.4
5–9 years	2	8	0.3	0.1–1.4
10+ years	5	12	0.5	0.2–1.5
Polycyclic aromatic hydrocarbons				
None	243	277	1.0	Referent
<5 years	13	23	0.7	0.3–1.3
5–9 years	12	8	1.8	0.7–4.7
10+ years	7	8	1.1	0.4–3.3

* Relative risk estimates are from logistic regression model with terms for duration of employment, race, age, parity, and gynecologic surgery.

† Exposure = number of years in the jobs assigned probabilities of definite, probable, and possible (P = 2–4) exposure to these chemicals.

Discussion

In this nonindustrial metropolitan area, working women typically held white-collar jobs with few exposures to known or suspected carcinogens. The possible role of exposures encountered in most manufacturing, agriculture, and other blue-collar occupations could not be evaluated. Nurses, waitresses, teachers, and office workers, on the other hand, were numerous, so any exposures occurring in those jobs could be assessed by the industrial hygienist. These jobs do not involve the level or variety of exposures often experienced by production jobs in manufacturing. Because no information was available on individual study subjects' exposure characteristics, it had to be assumed that exposures were homogeneous within job title (although it is known that this is not the case). Nonetheless, it is the typical occupational exposure assessment approach in most population-based case-control studies.

No indication of occupational hazard for ovarian cancer was seen in these data. This may have been because of the assumption of homogeneity or the small numbers. Nonetheless, it is still useful to have an industrial hygienist evaluate jobs for women. Women have held production jobs in manufacturing industries in World War II and today; many may experience exposures similar to men.[13] An industrial hygiene evaluation is particularly useful in case-control studies of cancers of the ovary or other sites for which there are seldom enough women in cohort studies to estimate occupational cancer risks accurately.

References

1. Frey CM. Ovary. In: Miller BA, Ries LAG, Hankey BF, Cosary CL, Edwards BK, eds., *Cancer Statistics Review, 1973–1989*. National Cancer Institute. NIH Publication No. 92-28789, 1992.
2. Whittemore AS, Harris R, Itnyre J, Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial cancers in white women. *Am J Epidemiol*. 1992;136:1184–1203.
3. Harris R, Whittemore AS, Itnyre J, Collaborative Ovarian Cancer Group. Char-

acteristics relating to ovarian cancer risk: collaborative analysis of twelve US case-control studies. III. Epithelial cancers of low malignant potential in white women. *Am J Epidemiol.* 1992;136:1204–1211.
4. Whittemore AS, Harris R, Itnyre J, Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. IV. The pathogenesis of epithelial ovarian cancer. *Am J Epidemiol.* 1992;136:1212–1220.
5. Harlow BL, Cramer DW, Bell DA, Welch WR. Perineal exposure to talc and ovarian cancer risk. *Obstet Gynecol.* 1992;80:19–26.
6. Whittemore AS, Wu ML, Paffenbarger RS Jr, et al. Personal and environmental characteristics related to epithelial ovarian cancer. II. Exposures to talcum powder, tobacco, alcohol, and coffee. *Am J Epidemiol.* 1988;128:1228–1240.
7. Hartge P, Schiffman MH, Hoover R, et al. A case-control study of epithelial ovarian cancer. *Am J Obstet Gynecol.* 1989;161:10–16.
8. Stewart PA, Herrick RF. Issues in performing retrospective exposure assessment. *Appl Occup Environ Hyg.* 1991;6:421–427.
9. International Agency for Research on Cancer. *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans.* Volume 46. Lyon, France: IARC; 1985.
10. Spirtas R, Stewart PA, Lee JS, et al. Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. *Br J Ind Med.* 1991;48:515–530.
11. Bureau of the Census. *1970 Census of Population, Alphabetic Index of Industries and Occupations.* Washington, DC: US Government Printing Office; 1971.
12. Rothman KJ. *Modern Epidemiology.* Boston: Little, Brown and Co; 1986, 38–40.
13. Stewart PA, Blair A. *Women in the formaldehyde industry: their exposures and their jobs.* J Occup Med 1994;36:918–923.

Exhibit 28

GYNECOLOGIC ONCOLOGY 45, 20–25 (1992)

Mineral Fiber Exposure and the Development of Ovarian Cancer

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A hospital-based case–control study of the association between fiber exposure and the development of epithelial ovarian cancer was performed at the Johns Hopkins Hospital in Baltimore, Maryland. Genital and respiratory fiber exposures were ascertained from incident cases (N = 77) and age–race matched controls (N = 46) using a structured questionnaire. Cases were ascertained between 1981 and 1985. An increased risk was observed for exposure to talc on sanitary napkins (OR = 4.79, 95% CI, = 1.29–17.79), genital fiber exposure from different sources for a long (cumulative exposure ≥37.4 years) length of time (OR = 2.35, 95% CI = 0.95–5.80), and occupational fiber exposure in relatives (OR = 2.81, 95% CI = 0.90–8.75). A negative association was observed for antecedent tubal ligation (OR = 0.15, 95% CI = 0.027–0.88). Findings from this study should be confirmed in larger investigations.

INTRODUCTION

Occupational exposure to asbestos has been identified as a risk factor for ovarian cancer in several studies [1–3]. Similarly, fiber-containing substances, such as talc, have also been implicated as risk factors [4–9]. A matched case–control study of epithelial ovarian cancer was conducted, in which the role of both genital and respiratory sources of fiber was assessed, to confirm these findings.

STUDY POPULATION

Cases and controls were ascertained from the Johns Hopkins Hospital between 1981 and 1985. This analysis is restricted to the 77 cases who were matched to 46 hospital controls and treated for conditions other than gynecologic or malignant diseases. Originally 140 newly diagnosed cases of epithelial ovarian cancer who met the eligibility criteria were ascertained from the Johns Hopkins Hospital. One hundred eight (77.1%) of these cases were successfully interviewed. These cases were diagnosed within 6 months of admission, were pathologically confirmed by examination of the ovaries, were admitted as in-patients for treatment or diagnosis, and were residents of the United States. Controls were in-patient females without gynecologic or malignant conditions who were initially matched to cases by age (within 5 years), race, and date of diagnostic admission (within 1 year). Since it was difficult to find controls meeting all of the matching criteria, a control could not be found for all cases. Unmatched cases were therefore matched a posteriori to controls, to form matched triplets of 2 cases and 1 control. A posteriori matching was performed within the same 5-year interval, by race and by closest date of diagnostic admission. The matching procedure allowed for the inclusion of 77 cases in the study. There were 46 matched sets, of which 31 consisted of 2 cases and 1 control. No matched control could be found for 13 cases, which were excluded from the analysis.

METHODS

Data were ascertained primarily from a questionnaire that was administered to participants both by telephone and in the hospital. Information on previous abdominal and gynecologic operations was also ascertained from medical records.

The questionnaire elicited information on the presence and length of genital fiber and respiratory fiber exposure, reproductive factors, estrogen use, family history of cancer in first-degree relatives, and other contraceptive use. Descriptions of the questions used to ascertain fiber exposure are listed in Appendix 1. Fiber exposure was defined as exposure to asbestos, talc (which may contain asbestos), and fiberglass.

An attempt was made to estimate the overall effect of

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"dose" or length of genital and respiratory fiber exposure. This was accomplished by adding the number of years of each type of genital or respiratory exposure from all sources. Since many of these exposures occurred at the same time, these variables should be considered as crude measures of dose rather than the true length of exposure.

Conditional logistic regression [10] was used to determine the strength of the association. Odds ratios were calculated to describe the relationship of genital and respiratory fiber exposure to ovarian cancer. The odds ratio is an estimate of the relative risk for relatively rare diseases such as ovarian cancer and, in this paper, is referred to as the relative risk estimate (RR).

The following variables were assessed as potential confounders:

Tobacco use
Number of cigarettes per day
Ovulatory time period
Number of pregnancies
Cancer in mother or father
Obesity 1 year prior to diagnosis
Obesity 20 years prior to diagnosis
Obesity at highest weight during the 20 years prior to diagnosis
Obesity according to average weight during the 20 years prior to diagnosis
Husband's and subject's education
Previous cancer
Marital status
Religion
Use of oral contraceptives
Use of contraceptive foams, creams, and jellies by themselves
Use of an IUD

On the basis of the strength of their relative risk estimates and the difference in frequency with which they were observed in cases and controls, the following variables were identified as potential confounders: measures of obesity, socioeconomic status (subject's education; RR = 0.5, 95% CI = 0.2–1.1), and religion (Jewish, RR = 2.9, 95% CI = 0.6–13.8; Catholic, RR = 0.5, 95% CI = 0.2–1.5), reproductive status (total live births, 1–2, RR = 0.7, 95% CI = 0.2–1.9; live births >2, RR = 0.4, 95% CI = 0.2–1.3), and oral contraceptive use (OR = 0.9, 95% CI = 0.3–23.0).

Obesity 1 year prior to diagnosis (RR = 2.3, 95% CI = 0.9–6.1), obesity 20 years prior to diagnosis (RR = 2.1, 95% CI = 0.7–6.9), and obesity according to highest weight during the 20 years prior to diagnosis (RR = 1.5, 95% CI = 0.7–3.2) were also found to be potential confounders. Obesity was defined as a height/weight index greater than that exhibited by women in the 85th percentile [11] of the population.

TABLE 1
Distribution of Matched Cases and Controls

	Cases		Controls	
	N	%	*N*	%
Age (years)				
Less than 30	3	3.9	2	4.4
30–39	1	1.3	1	2.2
40–49	11	14.3	8	17.4
50–59	21	27.3	10	21.7
60–69	35	45.4	21	45.6
70–79	5	6.5	3	6.5
80 or higher	1	1.3	1	2.2
Total	77		46	
Race				
White	70	90.9	41	89.1
Black	7	9.1	5	10.9
Total	77		46	

If a potential confounder changed the relative risk estimate associated with a fiber exposure by more than 15%, it was retained in the multivariate model. Confounders were sequentially added to multivariate models, depending on the level with which they changed the odds ratio of a fiber related exposure.

Interaction between fiber exposures and potential confounders was evaluated by comparing the deviance of models with and without the interaction term [12].

RESULTS

Age and Racial Distribution

Table 1 lists the age and racial distribution of cases and controls. Most cases and controls were in the age groups 40 to 69. It was difficult to obtain controls that did not have a chronic disease, resulting in a lower than expected number of controls.

Diagnoses of Controls

Controls were selected so that they did not have their primary diagnostic condition for more than 1 year. Table 2 lists the primary diagnosis of the controls included in the study.

Genital Fiber Exposure

Different sources of genital fiber exposure were examined (Table 3). Exposure from any of the potential sources of genital fiber was highly prevalent (91.1% in controls) and not found to be related to ovarian cancer (RR = 1.0, 95% CI = 0.2–4.0). Since it was felt that this index did not accurately characterize cumulative exposure, the median length of exposure from all genital sources (with the time since tubal ligation subtracted) was

TABLE 2
Distribution of Matched Controls by Primary Diagnosis

Diagnosis of restricted controls	*N*	%
Infectious and parasitic diseases	1	2.2
Diseases of the digestive system	6	13.0
Opthamologic disorders	15	32.6
Diseases of the circulatory system	11	23.9
Symptoms, signs, and ill-defined conditions	8	17.4
Diseases of musculoskeletal and connective tissue	1	2.2
Diseases of the endocrine system or metabolic or immunologic disorders	1	2.2
Diseases of the respiratory system	1	2.2
Benign neoplasms	1	2.2
Injury and poisoning	1	2.2
Total	46	100.00

used to categorize the dose of genital fiber exposure. A relative risk estimate of borderline significance was seen for exposure above the median length of time (37.4 years, RR = 2.4, 95% CI = 1.0–5.8). A significant negative association was observed for previous tubal ligation (RR = 0.2, 95% CI = 0.03–0.9).

A history of several gynecologic and abdominal operations, given by responses to the questionnaire, was examined to determine if exposure to talc from surgeon's gloves increased risk [13]. No statistically significant relationships were detected but, with the exception of ovarian biopsies, relative risk estimates were generally below 1. An attempt was made to combine information from the questionnaire and medical records, with no important changes in the relative risk estimates.

Moderately elevated relative risk estimates, which were not statistically significant, were observed with use of condoms, diaphragms (when powder was used), and genital bath talc. The level of association observed with exposure to talc on sanitary napkins (RR = 4.8, 95% CI = 1.3–18.0) was significantly greater than unity.

Respiratory Fiber Exposure

Numerous sources of respiratory fiber exposure were evaluated (Table 4): the only such source showing an important effect was occupational fiber exposure in relatives (RR = 2.8, 95% CI = 0.9–8.8). This relative risk estimate was calculated after three cases who were both exposed to asbestos themselves and received asbestos exposure from their relatives were excluded.

DISCUSSION

Genital talc exposure has been proposed as an etiologic agent of ovarian cancer [14] because talc was observed more frequently in cancerous ovaries [15] than in noncancerous ovaries, occupational studies of talc-containing asbestos showed an increased risk of lung cancer [16], asbestos has been observed in cosmetic face powders [17–19], and papillary growth has been observed after implantation of asbestos [20] and talc [21] into the peritoneal cavity.

We found an increased relative risk (4.8) for talc use on sanitary napkins with a smaller effect for genital bath talc exposure (RR = 1.7). This is in accordance with the original finding of a significant increased risk for perineal talc exposure (RR = 1.9, 95% CI = 1.3–2.9) by Cramer *et al.* [4]. Preliminary findings from a Chinese study also suggest that perineal application of talc-containing dusting powder increased the risk of epithelial ovarian cancer (RR = 3.9, 95% CI = 1.1–13.8) [5]. A nonsignificant effect for genital talc exposure (on genitals, sanitary napkins, or underwear) was detected in the study of Hartge *et al.* [6] (RR = 2.5, 95% CI = 0.7–10.0). Whittemore *et al.* [7] detected an increased risk (RR = 1.4, $P = 0.06$) for perineal exposure. In a study of borderline ovarian tumors, an increased risk was also observed with talc exposure from use on sanitary napkins (RR = 1.9, 95% CI = 0.9–6.9, 8).

We also investigated the relationship with cumulative duration of all forms of genital fiber exposure (with the time since tubal ligation subtracted). The positive association (RR = 2.35) in our study for exposure longer than the median length of time contrasts with the lack of association with duration observed by Whittemore *et al.* [7]. Whittemore *et al.* [7], however, did observe a positive dose–response relationship with frequency of exposure (1–20 times per month, RR = 1.3, 95% CI = 0.8–2.0; >20 times per month, RR = 1.4, 95% CI = 0.9–2.2). Although Booth *et al.*, [9] did not observe a significant ($P = 0.05$) trend of increasing risk with more frequent use, weekly genital talc use (RR = 2.0, 95% CI = 1.3–3.4) was associated with an increased risk of ovarian cancer.

The results of our study and others suggest that genital fiber exposure may be associated with an adverse effect [4–8] but further study is needed to determine if this relationship is causal in nature.

The present study also showed a significant negative association with tubal ligation (RR = 0.15). Harlow [22] (RR = 0.3, 95% CI = 0.3–1.1), Booth *et al.* [9] (RR = 0.2, 95% CI = 0.1–0.6), Mori *et al.* [23] (RR = 0.4, 95% CI = 0.2–1.0), Whittemore *et al.* [7] (RR = 0.6, $P = 0.07$), and Irwin *et al.* [24] (RR = 0.7, 95% CI = 0.5–1.0) also observed negative associations. Although the findings of our study differ from the positive association observed by Koch *et al.* [25] the comparison rates [26] used in Koch's cohort study may have been underestimated.

Tubal ligation may protect against ovarian cancer by inhibiting the carcinogenic action of talc through blockage

TABLE 3
Number and Frequency Distribution of Cases and Controls with Odds Ratios for Genital Fiber Exposure and Other Related Variables

Exposure interval	Attribute	Cases		Controls		Odds ratio	95% confidence interval
		N	%	*N*	%		
Genital fiber use	Yes	67	87.0	40	88.0	1.0	0.2–4.0[b]
	No	10	13.0	5	11.1		
	Missing	0		1			
Length of use of genital fiber[a] (years)	≥37.4	39	55.7	16	39.0	2.4	1.0–5.8[c]
	<37.4	31	44.3	25	61.0		
	Missing	7		5			
(Median of cases and controls)	Median		41.9		24.0		
Ovarian biopsies	Yes	6	7.8	3	6.5	1.1	0.3–4.4
	No	71	92.2	43	93.5		
Unilateral oophorectomy	Yes	8	10.4	5	10.9	0.8	0.2–2.5[d]
	No	69	89.6	41	89.1		
Tubal ligation	Yes	4	5.2	6	13.0	0.2	0.3–0.9[d,e]
	No	73	94.8	40	87.0		
Hysterectomy	Yes	19	24.7	12	26.1	0.7	0.3–1.7[d]
	No	58	75.3	34	73.9		
Condom use	Yes	35	49.3	22	51.2	1.6	0.6–3.9[b,d]
	No	37	50.7	21	48.8		
	Missing	5		3			
Diaphragm use with powder	Yes	14	18.9	5	11.4	3.0	0.8–10.8[b,d]
	No	60	81.1	39	88.6		
	Missing	3		2			
Genital bath talc	Yes	22	28.9	8	18.6	1.7	0.7–3.9
	No	54	71.0	35	81.4		
	Missing	1		3			
Sanitary napkin with talc exposure	Yes	21	30.0	6	13.6	4.8	1.3–17.8[f]
	No	49	70.0	38	86.4		
	Missing	7		2			

[a] After subtraction of the time since tubal ligation, for those who had ligation.
[b] Adjusted for number of live births.
[c] Adjusted for religion.
[d] Adjusted for years of education on subject.
[e] Adjusted for highest weight 20 years prior to diagnosis.
[f] Adjusted for highest weight 1 year prior to diagnosis.

of the fallopian tube or through a "screening" effect [27]. The effects of antecedent tubal ligation should be evaluated in future studies of ovarian cancer to determine if a negative association is consistently observed and to determine the reason for this.

Several cohort studies of women with respiratory exposure to asbestos detected an increased relative risk for ovarian cancer [1–3]. We elicited information about employment by relatives in occupations with asbestos or fiberglass exposure [28] and the relative risk was found to be elevated (RR = 2.8). In a previous case–control study of ovarian cancer, the relative risk for occupational asbestos exposure in relatives was not elevated [29], although the authors did not describe how the asbestos exposure was ascertained. Our initially suggestive findings regarding asbestos or fiberglass exposure in relatives should be evaluated further in additional studies.

In summary, our study shows that the development of ovarian cancer may be associated with genital fiber exposure (especially talc on sanitary napkins) and occupational exposure to fibers in relatives. Given its small sample size and the potential selection bias stemming from inclusion of patients from only one hospital, further research needs to be performed in order to confirm our findings.

APPENDIX 1

Questions Asked to Ascertain Fiber Exposures

Genital Fiber Exposure

1. Have you had any of the following operations prior to your hospitalization in ____?
   —Biopsy or removal of part of an ovary
   —Removal of one ovary
   —Removal of uterus (hysterectomy)

TABLE 4
Number and Frequency Distribution of Cases and Controls with Odds Ratios for Selected Characteristics Related to Respiratory Fiber Exposure

Exposure interval	Attribute	Cases		Controls		Odds ratio	95% confidence interval
		N	%	*N*	%		
Respiratory fiber exposure	Yes	69	89.6	41	89.1	1.3	0.3–3.6[a]
	No	8	10.4	5	10.4		
Cosmetic face powder use	Yes	38	50.7	24	54.5	1.1	0.4–2.7[b]
	No	37	49.3	20	45.4		
	Missing	2		2			
Insulation installed at residence	Yes	30	39.5	17	37.8	1.2	0.3–4.6[c]
	No	46	60.5	28	62.2		
	Missing	1		1			
Living in the vicinity of a fiber-emitting industrial establishment	Yes	7	9.2	4	8.9	1.0	0.3–3.4
	No	69	90.8	41	91.1		
	Missing	1		1			
Applied bath talc to body (respiratory exposure)	Yes	47	61.8	24	55.8	1.6	0.6–2.7[c]
	No	29	38.2	19	44.2		
	Missing	1		3			
Fiber exposure in relatives	Yes	18[d]	24.3	5	10.9	2.8	0.9–8.8
	No	56	75.7	41	89.1		
Use of spackling and taping compounds	Yes	20	29.0	14	31.1	1.6	0.5–3.4[a,e]
	No	49	71.0	31	68.9		
	Missing	8		1			

[a] Adjusted for highest weight 1 year prior to diagnosis.
[b] Adjusted for years of education on subject.
[c] Adjusted for number of live births.
[d] Three cases were excluded because they had been directly exposed to fibers and had a household member who had been occupationally exposed to fibers. Fiber exposure included asbestos, talc, or fiberglass exposure.
[e] Adjusted for religion.

—Tubal ligation
—Any other abdominal operation
(These items were confirmed by examination of medical records)

2. Did you use a diaphragm prior to your hospitalization in ____?

If yes: Did you use a powder to dust and dry the diaphragm?

If yes: Was it talc?

3. Did you and your sexual partner ever use a condom prior to your hospitalization in ____?

4. Have you regularly applied talcum powder to your body after bathing or for other reasons prior to your hospitalization in ____?

Did you commonly apply the talcum powder to your genital area?

5. Have you used talc on sanitary napkins or any other sanitary products used during your menstrual period prior to your hospitalization in ____?

Respiratory Fiber Exposure

1. Prior to your hospitalization in ____ had you ever regularly used cosmetic face powder?

2. Have you ever had insulation installed in a place that you lived in prior to your hospitalization in ____?

3. Have you ever lived in the vicinity of a shipyard, an asbestos or talc mine, or an asbestos, talc, or fiberglass processing plant prior to your hospitalization in ____?

4. Have you regularly applied talcum powder to your body after bathing or for other reasons prior to your hospitalization in ____?

Did you commonly apply the talcum powder to your face, upper torso, or legs and feet?

5. Have you or anyone who has ever lived in your household (including your husband) been employed in any of the following industries prior to your hospitalization in ____?

(a) Installation or removal of insulation materials
(b) Brake lining manufacture
(c) Automobile repair involving brake repair
(d) Roofing using asbestos materials
(e) Asbestos milling or mining
(f) Asbestos textile or paper manufacture
(g) Building construction
(h) Other industries where asbestos is used
(i) Talc mining and milling
(j) Other industries where talc is used
(k) Fiberglass or mineral wool manufacture
(l) Other industries where fiberglass or mineral wool was used

6. Did you ever use spackling and taping compounds?

If the respondent answered yes to any of these questions, they were asked when the exposure started and stopped. If they could not answer this question, they were asked how long the exposure occurred.

REFERENCES

1. Acheson, E. D., Gardner, M. J., Pipparh, E. C., and Grime, L. P. Mortality of two groups of women who manufactured gas masks from chrysotile and crocidolite asbestos: A 40 year follow-up, *Br. J. Ind. Med.* **29,** 344–348 (1982).
2. Newhouse, M. L., Berry, G., Wagner, J. C., and Turok, M. E. A study of the mortality of female asbestos workers, *Br. J. Ind. Med.* **29,** 134–141 (1972).
3. Wignall, B. K., and Fox, A. J. Mortality of female gas mask assemblers, *Br. J. Ind. Med.* **39,** 34–38 (1982).
4. Cramer, D. W., Welch, W. R., Scully, R. E., and Wojciechowski, C. A. Ovarian cancer and talc: A case–control study, *Cancer* **50,** 372–376 (1982).
5. Chen, Y., and Wu, B. Z. Risk factors for epithelial ovarian cancer in China—A case–control study (abstract), *Am. J. Epidemiol.* **132,** 777 (1990).
6. Hartge, P., Hoover, R., Lesher, L. P., and McGowan, L. Talc and ovarian cancer, *J. Am. Med. Assoc.* **250,** 1884 (1983).
7. Whittemore, A. S., Wu, M. L., Paffenbarger, R. S., Jr., Sarles, D. L., Kampert, J. B., Grosser, S., Jung, D. L., Ballon, S., and Hendrickson, M. Personal and environmental characteristics related to epithelial ovarian cancer. II. Exposures to talcum powder, tobacco, alcohol, and coffee, *Am. J. Epidemiol.* **128,** 1228–1240 (1988).
8. Harlow, B. L., and Weiss, N. S. A case–control study of borderline ovarian tumors. The influence of perineal exposure to talc, *Am. J. Epidemiol.* **30,** 390–394 (1989).
9. Booth, M., Beral, V., and Smith, P. Risk factors for ovarian cancer: A case–control study, *Br. J. Cancer* **60,** 592–598 (1989).
10. Smith, P. G., Pike, M. C., Hill, P. A., Breslow, N. E., and Day, N. E. Multivariate conditional logistic analysis of stratum-matched case–control studies, *Appl. Stat.* **30,** 190–197 (1981).
11. National Center for Health Statistics. Obese and overweight adults in the United States, in *National Health Survey Series No. 230,* Vol. 11, Hyattsville, MD. (1983)
12. Breslow, N. E., and Day, N. E. Statistical methods in cancer research, in *IARC scientific publications No. 32,* International Agency for Research on Cancer, Lyon (1980).
13. Tolbert, T. W., and Brown, J. L. Surface powders on surgical gloves, *Arch. Surg.* **115,** 729–732 (1980).
14. Longo, D. L., and Young, R. C. Cosmetic talc and ovarian cancer, *Lancet* **2,** 349–351 (1979).
15. Henderson, W. J., Joslin, C. A. F., Turnbull, A. C., and Griffiths, K. Talc and carcinoma of the ovary and cervix, *J. Obstet. Gynaecol. Br. Commonw.* **78,** 266–272 (1971).
16. International Agency for Research on Cancer. Silica and some silicates, in *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans,* Vol. 42, pp. 185–224, Lyons, France. (1987).
17. Cralley, L. J., Key, M. M., Groth, D. H., Lainhart, W. S., and Ligo, R. M. Fibrous and mineral content of cosmetic talcum products, *Am. Ind. Hyg. Assoc. J.* **29,** 350–354 (1968).
18. Paoletti, L., Caiazza, S., Donelli, G., and Pocchiari, F. Evaluation by electron microscopy techniques of asbestos contamination in industrial, cosmetic, and pharmaceutical talcs, *Reg. Toxicol. Pharmacol.* **4,** 222–235 (1984).
19. Rohl, A. N., Langer, A. M., Selikoff, I. J., Tordini, A., Klimentidis, R., Bowes, D. R., and Skinner, D. L. Consumer talcums and powders: Mineral and chemical characterization, *J. Toxicol. Environ. Health* **2,** 255–285 (1976).
20. Graham, J., and Graham, R. Ovarian cancer and asbestos, *Environ. Res.* **1,** 115–128 (1967).
21. Hamilton, T. C., Fox, H., Buckley, C. H., Henderson, W. J., and Griffith, S. K. Effect of talc on the rat ovary, *Br. J. Exp. Pathol.* **65,** 101–106 (1984).
22. Harlow, B. L. An epidemiologic study of borderline ovarian tumors, Doctor of Philosophy Dissertation, University of Washington (1987).
23. Mori, M., Harabuchi, I., Miyake, H., Casagrande, J. T., Henderson, B. E., and Ross, R. K. Reproductive, genetic, and dietary risk factors for ovarian cancer, *Am. J. Epidemiol.* **128,** 771–777 (1988).
24. Irwin, K., Weiss, N., Lee, N., and Peterson, H. Tubal sterilization, hysterectomy, and the subsequent occurrence of epithelial ovarian cancer (abstract), *Am. J. Epidemiol.* **132,** 777 (1990).
25. Koch, M., Starreveld, A. A., Hill, G. B., and Jenkins, H. The effect of tubal ligation on the incidence of epithelial cancer of the ovary, *Cancer. Detect. Prev.* **7,** 241–245 (1984).
26. Koch, M., Starreveld, A. A., Brown, L. B., and Gaedke, H. Survival trends in women with epithelial cancer of the ovary in Northern Alberta, 1960–1979, *Clin. Invest. Med.* **5,** 121–124 (1982).
27. Weiss, N. S., and Harlow, B. L. Why does hysterectomy without bilateral oophorectomy influence the subsequent incidence of ovarian cancer, *Am. J. Epidemiol.* **124,** 856–858 (1986).
28. Kusnetz, S., and Hutchison, B. K. *A guide to the work-relatedness of disease,* USDHEW, NIOSH, Cincinnatti, OH. (1979).
29. Newhouse, M. L., Pearson, R. M., Fullerton, J. M., Boesen, E. A. M., and Shannon, H. S. A case–control study of carcinoma of the ovary, *Br. J. Prev. Soc. Med.* **31,** 148–153 (1977).

Exhibit 29

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Risk Factors for Epithelial Ovarian Cancer in Beijing, China

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A study in Beijing, China of 112 pathologically confirmed epithelial ovarian cancer cases and 224 age-matched community controls enabled evaluation of risk in relation to reproductive, medical, familial, and selected lifestyle factors. An inverse relationship was observed between the number of full-term pregnancies and ovarian cancer risk. Compared to nulliparous women, subjects with one, two, or three full-term pregnancies were at 50%, 70%, or 90% reduced risks, respectively (P for trend <0.01). A positive correlation was found between the number of ovulatory years and risk, with a 2.6-fold increased risk for women with 30 or more compared to less than 10 ovulatory years (P for trend <0.01). Infertility, as estimated in various ways, was also found to be an important risk factor. When parity was taken into account, age at first pregnancy was not related to ovarian cancer risk. No protective effect was associated with mumps virus infection. In contrast, risk increased significantly as serum mumps virus antibody titres increased (P for trend <0.01). An elevated risk was found in women with a history of long-term (>3 months) application of talc-containing dusting powder to the lower abdomen and perineum (Relative risk 3.9, 95% confidence interval: 0.9–10.63). These findings suggest that Chinese women have risk factors similar to those of occidental women.

Although risk factors for ovarian cancer have been extensively studied in high-incidence areas, epidemiological patterns remain relatively unexplored elsewhere. In China, the incidence of ovarian cancer is much lower than in most western countries, with the incidence rate per 100000 women being 5.0 in Shanghai and 5.8 in Hong Kong[1] compared to 12.9 in Caucasian women in the San Francisco bay area.[1] However, ovarian cancer rates have been rising in China in recent years, and in one prospective study in Jiangsu Province ovarian cancer was found to account for 11.3% of all gynaecological malignancies, second only to the occurrence of cervical cancer.[2] Despite these rising incidence rates, the epidemiology of ovarian cancer in China remains relatively undefined, with only one analytical investigation having been previously undertaken in Shanghai.[3]

To assess the role of risk factors in China and to determine whether factors in this low-incidence area differ from those identified in other parts of the world, we conducted a case-control study in Beijing, China. Detailed personal interviews were undertaken, allowing an assessment of risk in relation to a variety of reproductive, medical, familial and other lifestyle factors.

MATERIAL AND METHODS

A total of 220 patients with newly diagnosed epithelial ovarian cancer occurring during the period 1984–1986 were identified through records at the Beijing Cancer Registry, a system designed to monitor all cancers in the Beijing metropolitan area. Records from the Registry were periodically checked against those of individual hospitals to assure completeness of the ascertainment mechanisms. After eliminating a large portion of cases because of death (n=67) or inability to locate n=37), 116 cases were included in the study group, of whom 112 were interviewed and four refused to cooperate. Many of the deaths occurred among the subjects diagnosed during the earliest year of the study, but deaths were not restricted to these patients. Because of the high nonresponse rate, we compared cases diagnosed during 1984 to the other cases and found no major differences with respect to the major identified menstrual, reproductive and medical risk factors. Results from the entire data set are presented here.

Diagnosis was confirmed by laparotomy and pathological examination in all 112 cases, with serous cancer

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accounting for 51% (57 cases), mucinous cancer for 19% (21 cases) and miscellaneous epithelial cancer for 30% (34 cases).

For each interviewed case in the study, two population controls were selected and interviewed within a short period of the matched case. Depending on whether the case resided in an urban or a rural area, the first unit of selection was the same street office or township (commune) as that of the case. Within these areas, a random selection was made of one neighbourhood committee or village, which was visited to abstract information from census lists of all women who were within 1 year of age of the identified case. We excluded women who had ever had a serious illness (including gynaecological diseases and a variety of abdominal abnormalities) from these potential controls. A random number table was then used to select two controls for each ovarian cancer patient. A total of 15 of the initially selected controls refused to co-operate, and were replaced with another eligible control. A total of 224 controls were interviewed.

A comprehensive questionnaire was developed with special emphasis on menstrual, obstetric, marital, medical, familial and dietary histories. All exposure information sought was with reference to events occurring 3 or more years prior to the date of diagnosis (equivalent date in controls). Data were collected through face-to-face interviews by trained interviewers. All interviews were tape recorded and checked by the authors. Peripheral blood samples were taken for determination of ABO blood groups and mumps virus antibody titres.

The odds ratio was used as an estimate of relative risk (RR). Logistic regression for matched sets was used to control for potential confounding effects of selected variables and to obtain maximum likelihood estimates of relative risks and 95% confidence intervals (CI).[4] Tests for trend were computed by treating the categorical variables as continuous variables in the regression models.[4]

RESULTS

The mean age at interview was 48.5 years among cases and 49.0 years among controls. The cases tended to be more educated than controls, with relative risks of 1.8 for senior high school graduates and 3.2 for those who received higher education compared to those who had no formal education (P for trend <0.01) (Table 1). Because of this discrepancy, it was necessary to adjust for effects of education when evaluating the risk associated with related factors. Cases and controls were similar with respect to income, average weight, and height.

TABLE 1 *Descriptive social factors for ovarian cancer cases versus controls*

	Cases	Controls	RR	95% CI
Education				
None	29	62	1.0	
Primary	22	58	0.8	0.4–1.7
Junior high school	18	48	0.9	0.4–2.2
Senior high school	23	37	1.8	0.7–4.5
College	20	19	3.2	1.2–8.4
			P for trend	<0.01
Income[a] (yuan)				
<500	53	134	1.0	
500–999	54	78	1.8	1.1–3.0
1000+	5	12	1.0	0.3–3.1
			P for trend	=0.37
Weight (kg)				
<50	22	41	1.0	
50–59	41	94	0.8	0.4–1.5
60–69	34	63	1.0	0.5–2.0
70+	15	26	1.1	0.5–2.6
			P for trend	=0.45
Height (cm)				
<155	21	33	1.0	
155–159	35	88	0.6	0.3–1.3
160–164	36	78	0.7	0.4–1.4
165+	20	25	1.3	0.6–3.1
			P for trend	=0.55

[a] Yearly income (1 US$ = 3.7 yuan)

As with previous studies, a strong inverse relationship was observed between gravidity or parity and risk of ovarian cancer (Table 2). Relative to the 20 patients and 23 controls who had never given birth, the risk was 0.5 for women who had one birth and 0.3 for those who had two births. A ten-fold reduction in risk was obtained by the third birth. The trend persisted after adjustment for education. A similar relationship was observed for number of pregnancies. In the univariate analyses, late age at first pregnancy emerged as a strong factor, but its effects disappeared after adjustment for parity. Duration of breastfeeding was similarly inversely related to risk, but this effect also disappeared after adjustment for parity. Induced abortions and miscarriages appeared to decrease risk but their trends were not statistically significant. A previous stillbirth was reported by two cases and four controls, resulting in a RR of 1.9 (95% CI: 0.3–11.5) after adjustment for education and parity. Five cases and five controls had a history of caesarean section (RR = 1.2, 95% CI: 0.3–5.1).

Parity or gravidity partly reflect fertility, but are also strongly influenced by voluntary contraception.

TABLE 2 *Reproductive factors and risk of ovarian cancer*

	Cases	Controls	RR[a]	95% CI
Gravidity				
0	17	22	1.0	
1	18	14	1.1	0.4–3.8
2	19	22	0.5	0.1–1.9
3	16	38	0.2	0.1–0.9
4–5	23	66	0.2	0.1–0.8
6+	19	62	0.2	0.0–0.7
Trend test			$P<0.01$	
Parity				
0	20	23	1.0	
1	28	38	0.5	0.2–1.8
2	27	45	0.3	0.1–1.2
3	12	34	0.1	0.0–0.6
4–5	17	57	0.1	0.0–0.5
6+	8	27	0.1	0.0–0.6
Trend test			$P<0.01$	
Age at first pregnancy[b,c] (years)				
<20	19	43	1.0	
20–24	34	88	0.7	0.3–1.5
25–29	29	62	0.4	0.2–1.2
30+	13	9	1.1	0.2–4.6
Trend test			$P=0.65$	
Breastfeeding[c] (years)				
0	39	49	1.0	
<1	11	15	0.9	0.3–2.9
1	13	24	0.8	0.3–2.2
2	12	25	0.9	0.3–3.0
3+	37	111	1.1	0.4–2.9
Trend test			$P=0.71$	
Induced abortion[c]				
0	71	120	1.0	
1	24	61	0.8	0.4–1.5
2	12	26	0.8	0.4–1.9
3+	5	17	0.5	0.2–1.6
Trend test			$P=0.39$	
Miscarriage[c]				
0	97	179	1.0	
1	11	35	0.4	0.2–1.1
2+	4	10	0.9	0.2–3.6
Trend test			$P=0.64$	

[a] Adjusted for education
[b] Among gravid women only, unknowns excluded
[c] Further adjusted for parity

TABLE 3 *Fertility factors and risk of ovarian cancer*

	Cases	Controls	RR[a]	95% CI
Fertility index[b]				
0	11	11	1.0	
<0.25	33	44	0.6	0.2–2.1
0.25–0.49	31	71	0.3	0.1–1.0
0.5–0.99	17	43	0.2	0.1–0.8
1+	11	38	0.1	0.0–0.3
Trend test			$P<0.01$	
Nulligravidae[c]				
Unmarried	7	15	1.0	
Married	10	7	5.7	0.4–80.9
No trouble conceiving	2	3	1.0	
Had trouble conceiving	8	4	5.6	0.4–81.1
Gravid women[c]				
No trouble conceiving	83	181	1.0	
Had trouble conceiving	12	21	1.3	0.6–2.8
Ovulatory years				
<10	10	21	1.0	
10–19	27	74	0.8	0.2–2.9
20–29	44	97	1.3	0.3–5.5
30+	31	32	2.6	0.6–11.2
Trend test			$P=0.01$	

[a] Adjusted for education
[b] Among married women only and calculated as the number of pregnancy/(age at menopause – age at marriage – duration of separation – duration of contraception – time interval between sterilization and menopause)
[c] Using unmatched stratified analysis and adjusted for education and age. Trouble conceiving was defined as married woman who tried to conceive but failed for a period of more than 1 year.

Thus, a fertility index was introduced in order to more accurately estimate fertility (Table 3). This was calculated as the number of pregnancies divided by the total number of years of potential pregnancy. Years of potential pregnancy was calculated as age at menopause (or age at diagnosis for premenopausal cases and their matched controls) minus age at marriage minus duration of separation minus duration of contraception minus time interval between sterilization and menopause. As in Table 3, risk decreased as the index increased, with an effect as strong as that of parity. Married nulligravidae had a 5.7-fold increase in risk compared to single nulligravidae (95% CI: 0.4–80.9). Risk was also elevated among married nulligravidae with a history of trouble conceiving for 1 or more years (RR = 5.6, 95% CI: 0.4–81.1). Among gravid women, 12 cases versus 21 controls reported a history of trouble conceiving (RR = 1.3, 95% CI: 0.6–2.8).

We also examined the effect of total duration of ovulation on risk of ovarian cancer to test the hypothesis that incessant ovulation is associated with increased risk. Total ovulatory years were calculated as age at menopause minus age at menarche minus duration of amenorrhoea minus duration of oral contraceptive use. Risk increased significantly with years of ovulation, with the RR rising from 1.3 (95% CI: 0.3–5.5) to 2.6 (95% CI: 0.6–11.2) for those with 20–29 and 30 or more years of ovulation, respectively, com-

pared to less than 10 years of ovulation. This effect may be entirely due to the low parity among women with 20 or more ovulatory years, but the high correlation between these two variables prevented further disentanglement of effects.

The associations of some selected menstrual and hygienic factors are summarized in Table 4. Age at menarche appeared unrelated to risk. Among naturally menopausal women, age at menopause appeared to exert no effect on risk. Eight cases and 22 controls reported irregular menstruation during most of their lives, which was associated with a RR of 1.3 (95% CI: 0.5–3.2). Dysmenorrhoea, or abdominal pain during the first 3 days of menses, was not related to any significant alteration in risk (RR = 0.7, 95% CI: 0.4–1.2). Premenstrual tension, defined as the presence of three or more premenstrual symptoms (irritability, anxiety, headache, sleepless, breast tenderness, oedema etc.) also did not significantly affect risk. Seven cases and five controls reported using dusting powder to the lower abdomen and perineum for 3 or more months (RR = 3.9, 95% CI: 0.9–10.6).

TABLE 4 *Menstruation and hygiene and risk of ovarian cancer*

	Cases	Controls	RR[a]	95% CI
Age at menarche (years)				
< 14	28	51	1.0	
14–15	45	104	1.1	0.6–2.0
16–17	36	47	2.1	0.9–4.4
18+	3	22	0.4	0.1–1.8
Trend test			$P = 0.55$	
Age at menopause[b]				
<45	6	24	1.0	
45–49	25	49	1.1	0.4–3.5
50+	24	45	1.0	0.3–3.4
Trend test			$P = 0.56$	
Menstrual irregularity				
No	104	202	1.0	
Yes	8	22	1.3	0.5–3.2
Dysmenorrhoea				
No	63	121	1.0	
Yes	49	103	0.7	0.4–1.2
Premenstrual tension				
No	74	138	1.0	
Yes	38	86	0.6	0.4–1.1
Dusting powder				
No	105	219	1.0	
Yes	7	5	3.9	0.9–10.6

[a] Adjusted for education and parity.
[b] Among naturally menopausal women only, unknowns excluded.

Table 5 presents the association between birth control methods and risk of ovarian cancer. Women who took oral contraceptives for less than 1 year had a nonsignificantly reduced risk compared to nonusers (RR = 0.7, 95% CI: 0.3–1.8). However, the risk appeared to be elevated for subjects who used oral contraceptives for longer periods of time, with the RRs being 1.4 (95% CI: 0.5–3.4) for 1–2 years and 1.1 (95% CI: 0.4–2.9) for 3 years or more of usage. However, the trend of risk with duration of oral contraceptive use was not statistically significant (P for trend = 0.75). No significant association was noted between use of condoms or intrauterine devices (IUD) and risk, but IUD use was accompanied by a nonsignificant reduction in risk of ovarian cancer. A total of 11 cases and 36 controls reported prior sterilization, but this was not associated with any alteration in risk.

TABLE 5 *Use of different contraceptive methods and risk of ovarian cancer*

	Cases	Controls	RR[a]	95% CI
Oral contraceptives (months)				
0	81	153	1.0	
<12	9	30	0.7	0.3–1.8
12–35	12	20	1.4	0.5–3.4
36+	10	21	1.1	0.4–2.9
Trend test			$P = 0.75$	
Condom (months)				
0	72	132	1.0	
<12	9	26	0.8	0.3–2.1
12–35	8	24	0.5	0.2–1.3
36+	23	42	0.9	0.4–2.0
Trend test			$P = 0.58$	
Intrauterine device (months)				
0	89	153	1.0	
<12	4	13	0.6	0.2–2.0
12–35	4	17	0.3	0.1–1.3
36+	15	41	0.6	0.3–1.4
Trend test			$P = 0.21$	
Sterilization				
No	101	188	1.0	
Yes	11	36	1.0	0.5–2.3

[a] Adjusted for education and parity.

Several hereditary factors were also examined. (Table 6). Blood group did not appear to play a major aetiological role. Subjects were questioned regarding the age of their mother when they were born and the number of children borne by their mother, but neither of these was found to be a predictor of ovarian cancer

TABLE 6 *Hereditary factors and risk of ovarian cancer*

	Cases	Controls	RR[a]	95% CI
Blood groups				
O	43	74	1.0	
A	28	56	0.9	0.5–1.7
B	30	69	0.8	0.4–1.4
AB	11	25	0.6	0.3–1.5
Mother's age at which the subject was born				
<30	71	116	1.0	
30–39	28	84	0.5	0.3–0.9
40+	9	20	0.9	0.4–2.5
Unknown	4	4	1.5	0.3–8.2
Trend test			$P=0.42$	
Number of children borne by the subject's mother				
1–3	22	40	1.0	
4–6	49	93	0.9	0.5–1.7
7+	40	90	0.8	0.4–1.5
Unknown	1	1		
Trend test			$P=0.91$	
Familial malignancies (mainly intestinal tumours)				
No	95	204	1.0	
Yes	17	20	1.9	0.9–4.3
Tuberculosis in family members				
No	93	190	1.0	
Yes	19	34	0.8	0.4–1.7

[a] Adjusted for education and parity.

risk. A positive history of familial malignancies, most of which were gastrointestinal tumours, was noted for 17 cases and 20 controls, resulting in a RR of 1.9 (95% CI: 0.9–4.3). Tuberculosis in family members was not related to risk.

Contrary to an earlier report, no protective effect of mumps virus infection was observed in this study, with 30 patients versus 69 controls reporting such a history (RR = 0.9, 95% CI: 0.5–1.5). Furthermore, the mumps antibody titres of cases were significantly higher than those of controls. Compared to those with an antibody titre less than 1 : 5, women with a titre of 1 : 5 had a RR of 0.7 (95% CI: 0.3–1.8) and the same risk was also found for women with an antibody titre of 1 : 10 (RR = 0.7; 95% CI: 0.3–1.7). Risk increased to 1.8 (95% CI: 0.8–3.9) for women with an antibody titre of 1 : 20 and 3.6 (95% CI: 1.6–8.1) for those with a 1 : 40 titre. The trend remained statistically significant after adjustment for education and parity (P for trend = 0.002).

Occupational exposures to talc, asbestos and heavy metals were associated with RRs of 0.9 (95% CI: 0.3–2.9), 0.7 (95% CI: 0.3–1.5) and 2.1 (95% CI: 0.7–6.4), respectively.

A total of 19.6% of the cases were smokers compared with 24.6% of the controls (RR = 0.9, 95% CI: 0.4–1.7).

COMMENTS

The findings of this study must be interpreted in light of three methodological limitations. Given the nature of cancer registration in China, some ovarian cancer patients may not have been ascertained for study, despite efforts to obtain a complete series by additionally reviewing hospital records. The extent to which any losses might have affected results is unclear, but if those subjects not identified differed significantly from those included some risks could have been over- or underestimated. Potentially more damaging was the high rate of loss due to deaths. Since the case group contained a disproportionate share of survivors, risk estimates could reflect influences on survival as well as risk. However, it would appear that these potential biases may have been relatively minor, since risk factors identified were of a similar magnitude to those from investigations involving complete incident case series. In addition, separate analyses of the earlier versus later cases showed similarities with respect to education, parity, age at first pregnancy, ovulatory years, mumps antibody titre, and occupational associations. A third limitation was the exclusion of controls with current health problems. This exclusion should not have affected the results pertaining to childhood mumps or family cancer history, since it is unlikely that these conditions affected current health. However, it is possible that the exclusions could have affected the assessment of other medical conditions, e.g., thyroid problems.

For these reasons, some caution must be exercised in the interpretation of certain results. However, since there is a paucity of data on ovarian cancer risk factors among women in low-risk countries, the results are of interest, particularly when compared with studies in other parts of the world. Numerous epidemiological studies have been conducted elsewhere to explore risk factors for ovarian cancer, but the aetiology of ovarian cancer remains largely unknown. The most consistent finding across studies has been a protective effect of pregnancy,[5–11] a relationship also confirmed in the present investigation.

Several hypotheses have been suggested to explain the protective effect of higher parity. Fathalla[12] suggested that incessant ovulation might initiate ovarian

cancer and that pregnancy might exert its protective effect by preventing ovulation. Alternatively, it has been suggested that pregnancy inhibits the secretion of pituitary gonadotropins, thereby reducing the risk of ovarian cancer.[13] Finally, it is possible that an unknown factor leads to both low parity and ovarian cancer.

If pregnancy protects against ovarian cancer through its inhibition of ovulation, one would expect that any factor that deters ovulation would reduce risk. Furthermore, the magnitude of protection should be determined by the effectiveness of the factor in preventing ovulation and the duration of exposure to the factor. Thus, every factor involved in the calculation of ovulatory years should affect risk of ovarian cancer, including age at menarche, age at menopause, duration of breastfeeding, and duration of amenorrhoea.

Results from this study do not support the hypothesis of a single underlying effect of ovulation. Consistent with several studies,[14-16] we found no relationship of risk to either age at menarche or age at menopause. Furthermore, we did not observe an effect of oral contraceptive use on risk of ovarian cancer, although the low prevalence of exposure to oral contraceptives in our study (32% among controls) limited the power to detect an effect. Several investigations have also noted no association between oral contraceptive use and risk of ovarian cancer[3,5,14] although strong evidence for a protective effect has been observed in many studies.[7-10]

The long duration of breastfeeding among Chinese women provided us with a unique opportunity to evaluate risk of ovarian cancer with respect to lactation. Reduced risk with increased years of breastfeeding, seen in the univariate analysis, disappeared with adjustment for the effect of parity. This finding is in agreement with other studies.[17-20]

Of the four factors involved in the calculation of ovulatory years, only duration of amenorrhoea was related to risk. However, since ovulatory years is highly dependent on parity and breastfeeding, its relationship to risk may merely be an alternative measure of parity.

In this study, ovarian cancer risk was increased among those with a low fertility index. It was impossible to distinguish clearly the effects of this from those associated with parity, but it is noteworthy that married nulliparas had a 5.7 times higher risk than single nulliparas, and that those who tried but failed to conceive for more than 1 year had a five-fold increased risk. Several other investigations[5,13,14,20] have found a link between infertility and ovarian cancer risk, but it is difficult to accurately assess fertility in case-control studies. On the other hand, a prospective follow-up study of infertile women, with laboratory tests and clinical surveillance, would have to be extremely large to detect any effects of infertility on risk of development of ovarian cancer.

Late age at first pregnancy has been suggested as a possible risk factor for ovarian cancer,[21] but confounding effects of parity must be carefully considered. In this, and most other studies,[14,17,20,22] age at first pregnancy does not affect risk after adjustment for parity and education.

Cramer *et al.*[23] suggested that mumps infection may cause oophoritis, leading to oocyte depletion and initiation of cancerous changes in ovarian epithelium. Although Menczer *et al.*[24] found that cases less frequently reported histories of mumps infection, we, similar to another study,[25] observed no differences between cases and controls. In addition, we found exactly the opposite of Menczer, who reported lower serum antibody titres in cases than controls. Thus, our results provide little evidence for a protective effect of mumps virus infection on subsequent ovarian cancer risk.

We additionally investigated several sources of potential talc exposure. Among these, the only exposure that seemed to increase ovarian cancer was hygienic practices involving use of dusting powder on the lower abdomen and perineum. Similar to previous studies,[26,27] a threefold increased risk was associated with this practice, but other exposures that might bring talc into the pelvic cavity, such as abdominal surgery, pelvic examination (through medical gloves) and occupational exposures were not associated with risk. The small number of women who used dusting powder (seven cases and five controls) makes it impossible to distinguish among the types of exposures. It is nonetheless interesting that similar results have been obtained from quite different parts of the world, leading to the conclusion that the relationship of talc to ovarian cancer risk deserves further study.

Despite the potential shortcomings of the methodology of this study, the data appear to be valid, for example, in showing the same parity effect seen elsewhere in the world. In general, these data show that risk factors for Chinese women are strikingly similar to those found elsewhere, despite great differences in incidence rates between China and most western countries.

REFERENCES

[1] Muir C, Waterhouse J, Mack T, *et al. Cancer Incidence in Five Continents, Vol. 5.* IARC Scientific Publication 88. Lyon, France: IARC, 1987.

[2] Liu B L. Epidemiologic Study of Gynecologic Malignancy in Jiangsu Province. *Chin Med J* (English Edition) 1984; **97:** 711–16.

[3] Shu X O, Brinton L A, Gao Y T, *et al.* Population-based case-control study of ovarian cancer in Shanghai. *Cancer Res* 1989; **49:** 3670–74.

[4] Lubin, J. A computer program for the analysis of matched case-control studies. *Comput Biomed Res* 1981; **14:** 138–43.

[5] Casagrande J T, Pike M C, Ross R K, *et al.* 'Incessant ovulation' and ovarian cancer. *Lancet* 1979; **ii:** 170–73.

[6] Newhouse M L, Pearson R M, Fullerton J M, *et al.* A case control study of carcinoma of the ovary. *Br J Prev Soc Med* 1977; **31:** 148–53.

[7] Risch H A, Weiss N S, Lyon J L, *et al.* Events of reproductive life and the incidence of epithelial ovarian cancer. *Am J Epidemiol* 1983; **117:** 128–39.

[8] Cramer D W, Hutchison G B, Welch W R, *et al.* Factors affecting the association of oral contraceptives and ovarian cancer. *N Engl J Med* 1982; **307:** 1047–51.

[9] Hildreth N G, Kelsey J L, LiVolsi V A, *et al.* An epidemiologic study of epithelial carcinoma of the ovary. *Am J Epidemiol* 1981; **114:** 398–405.

[10] Franceschi S, La Vecchia C, Helmrich S P, *et al.* Risk factors for epithelial ovarian cancer in Italy. *Am J Epidemiol* 1982; **115:** 714–19.

[11] Nasca P C, Greenwald P, Chorost S, *et al.* An epidemiologic case-control study of ovarian cancer and reproductive factors. *Am J Epidemiol* 1984; **119:** 705–13.

[12] Fathalla M F. Factors in the causation and incidence of ovarian cancer. *Obstet Gynecol Surv* 1972; **27:** 751–68.

[13] Joly D J, Lilienfeld A M, Diamond E L, *et al.* An epidemiologic study of the relationship of reproductive experience to cancer of the ovary. *Am J Epidemiol* 1974; **99:** 190–209.

[14] McGowan L, Parent L, Lednar W, *et al.* The woman at risk for developing ovarian cancer. *Gynecol Oncol* 1979; **7:** 325–44.

[15] Annegers J F, Strom H, Decker D G, *et al.* Ovarian cancer: Incidence and case-control study. *Cancer* 1979; **43:** 723–29.

[16] Hartge P, Hoover R, McGowan L, *et al.* Menopause and ovarian cancer. *Am J Epidemiol* 1988; **127:** 990–98.

[17] Cramer D W, Hutchison G B, Welch W R, *et al.* Determinants of ovarian cancer risk. I. Reproductive experiences and family history. *JNCI* 1983; **71:** 711–16.

[18] Mori M, Harabuchi I, Miyake H, *et al.* Reproductive, genetic, and dietary risk factors for ovarian cancer. *Am J Epidemiol* 1988; **128:** 771–77.

[19] Booth M, Beral V, Smith P. Risk factors for ovarian cancer: A case-control study. *Br J Cancer* 1989; **60:** 592–98.

[20] Hartge P, Schiffman M H, Hoover R, *et al.* A case-control study of epithelial ovarian cancer. *Am J Obstet Gynecol* 1989; **161:** 10–16.

[21] La Vecchia C, Decarli A, Franceschi S, *et al.* Age at first birth and the risk of epithelial ovarian cancer. *JNCI* 1984; **73:** 663–66.

[22] Wu M L, Whittemore A S, Paffenbarger R S Jr, *et al.* Personal and environmental characteristics related to epithelial ovarian cancer. I. Reproductive and menstrual events and oral contraceptive use. *Am J Epidemiol* 1988; **128:** 1216–27.

[23] Cramer D W, Welch W R, Cassells S, *et al.* Mumps, menarche, menopause, and ovarian cancer. *Am J Obstet Gynecol* 1983; **147:** 1–6.

[24] Menczer J, Modan M, Ranon L, *et al.* Possible role of mumps virus in the etiology of ovarian cancer. *Cancer* 1979; **43:** 1375–79.

[25] Schiffman M H, Hartge P, Lesher L P, *et al.* Mumps and postmenopausal ovarian cancer. *Am J Obstet Gynecol* 1985; **152:** 116–17.

[26] Cramer D W, Welch W R, Scully R E, *et al.* Ovarian cancer and talc: A case-control study. *Cancer* 1982; **50:** 372–76.

[27] Whittemore A S, Wu M L, Paffenbarger R S Jr, *et al.* Personal and environmental characteristics related to epithelial ovarian cancer. II. Exposures to talcum powder, tobacco, alcohol, and coffee. *Am J Epidemiol* 1988; **128:** 1228–40.

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Exhibit 30

Int. J. Cancer: **55**, 408–410 (1993)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

HAIR DYES, ANALGESICS, TRANQUILIZERS AND PERINEAL TALC APPLICATION AS RISK FACTORS FOR OVARIAN CANCER

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In a hospital-based case-control study of ovarian cancer conducted in Athens (1989–1991), 189 women with histologically confirmed common malignant epithelial tumors of the ovary were compared with 200 hospital visitor controls. All interviews were conducted by personal interview in the 2 participating hospitals and the data were analyzed by modelling through logistic regression, controlling for demographic and reproductive variables. Tranquilizing and hypnotic drugs (mostly diazepam) were not associated with risk of ovarian cancer: the adjusted relative risk (RR) and 95% confidence interval (CI) were 0.96 (0.57 to 1.63), whereas use of analgesics (mostly salicylates) was associated with significantly reduced risk of the disease (RR 0.51; CI 0.26 to 1.02). There was no evidence that perineal application of talc was associated with increased risk (RR 1.05; CI 0.28 to 3.98) but the frequency of reporting talc use was low in the study population. There was a statistically significant (*p* for trend 0.007) and a dose-dependent association between hair dyeing and risk of ovarian cancer. Compared to never-users, women dyeing their hair up to 4 times per year had a relative risk of 1.74 (0.91 to 3.32) whereas those dyeing their hair 5 or more times per year had a relative risk of 2.16 (1.19 to 3.89).

Ovarian cancer is an important cause of morbidity and mortality in most populations. The prognosis of the disease is poor and has improved little over the last 30 years. Several epidemiologic studies exploring the etiology of ovarian cancer have been undertaken and, recently, 2 major meta-analyses have summarized the results of European (Franceschi *et al.*, 1991*a,b;* Negri *et al.*, 1991) and American (Whittemore *et al.*, 1992*a,b,c*) studies. The only consistent findings were the inverse associations of ovarian cancer risk with parity and oral contraceptive use (Negri *et al.*, 1991; Franceschi *et al.*, 1991*b;* Hankinson *et al.*, 1992; Whittemore *et al.*, 1992*a*); these associations cannot explain the almost 3-fold international variation in the incidence of ovarian cancer.

In a case-control study undertaken in Athens in 1989–1991 we have examined, in addition to reproductive variables and tobacco, coffee and ethanol consumption (Polychronopoulou *et al.*, 1993) the possible importance, as ovarian cancer risk factors, of hair dyes, analgesics, tranquilizers and perineal talc application. The evidence concerning the potential involvement of these factors in the etiology of ovarian cancer is limited, speculative, or indirect. In this paper we report the results of our study with respect to these factors.

SUBJECTS AND METHODS

The characteristics of the study subjects have been described (Polychronopoulou *et al.*, 1993). Briefly, cases were 189 women residents of Greater Athens and under 75 years of age (90% of those eligible), who underwent surgery for a common epithelial ovarian tumor in the 2 major cancer hospitals of the Greater Athens area between June 1989 and March 1991, whereas controls were 200 women, residents of the Greater Athens area and under 75 years of age, visiting patients hospitalized in the same wards as the cancer patients at the same time (94% of those eligible). The women were interviewed in the hospital wards by 2 medical residents of the respective hospitals.

Cases and controls were also asked to report the frequency of use (over an extended period before the onset of the present disease for cases, or a comparable period before the interview for controls) of analgesics (never or rarely; infrequently, *i.e.* once per week or less; and frequently, *i.e.* twice per week or more, on the average), tranquilizers or hypnotics (never or rarely; frequently), hair dyes (never; up to 4 times per year; 5 or more times per year) and talc in the perineal region (no; yes). Until recently, when acetaminophen-based drugs became popular, most over-the-counter analgesics in Greece were based on salicylates, whereas diazepam-based drugs have dominated the tranquilizer market in Greece (the distinction between tranquilizers and hypnotics was not easily apparent to most women, and both categories were in the past frequently sold without prescription).

The analysis was done first after stratification by age in 10-year groups and then by modeling the data through multiple logistic regression using the SAS statistical package. In the multivariate analysis the following variables were controlled for, on account of their possible importance as selection or confounding factors: age (in 5-year groups); years of schooling (0–5, 6–11, 12+ years); weight before onset of the disease (in 5-kg, continuously); age at menarche (in single years, continuously); menopausal status (pre-menopausal, menopausal including climacteric); for post-menopausal and climacteric age at menopause (in 5-years, continuously); parity (nulliparous, parous); for parous age at first birth (in 5-years, continuously); tobacco smoking (non-smokers, ever-smokers); average consumption of alcoholic beverages (in glasses/day, continuously); and average coffee drinking (in cups/day, continuously). The 4 variables examined in this study (use of analgesics, tranquilizers, hair dyes and talc in the perineal region) were also simultaneously introduced in the multivariate model, although there was virtually no mutual confounding among them.

RESULTS

The results concerning analgesics and tranquilizers/hypnotics are presented in Table I. There is clearly no evidence that either analgesics or tranquilizers/hypnotics increase the risk of ovarian cancer. On the contrary, after adjustment for a set of probable confounding factors, the inverse association with frequency of use of analgesics becomes marginally significant with an apparent dose-response trend.

Table II shows frequency distributions as well as crude and adjusted rate ratios associated with the practice and frequency of hair dyeing and the local application of talc in the perineal region. Although the number of talc users is in general small

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TABLE I – REPORTED USE OF ANALGESICS AND TRANQUILIZERS/HYPNOTICS BY 189 WOMEN WITH OVARIAN CANCER (COMMON EPITHELIAL TUMORS) AND 200 COMPARISON WOMEN. RELATIVE RISK POINT ESTIMATES (95% CONFIDENCE INTERVALS)

	Use of analgesics			*p*-value for trend (1 d.f.)
	Never/rarely	Infrequently	Frequently	
Cases	43	102	44	
Controls	35	112	53	
RR (crude)	1	0.74 (0.44–1.25)	0.68 (0.37–1.23)	0.21
RR (age-adjusted)	1	0.93 (0.53–1.63)	0.61 (0.32–1.17)	0.11
RR (multiple regression)[1]	1	0.78 (0.43–1.43)	0.51 (0.26–1.02)	0.05
	Use of tranquilizers/hypnotics			*p*-value (1 d.f.)
	No	Yes		
Cases	143	46		
Controls	146	54		
RR (crude)	1	0.87 (0.55–1.37)		0.55
RR (age-adjusted)	1	0.87 (0.53–1.42)		0.58
RR (multiple regression)[1]	1	0.96 (0.57–1.63)		0.89

[1]Controlling for age, years of schooling, weight before onset of the disease, age at menarche, menopausal status and age at menopause, parity and age at first birth, tobacco smoking, coffee drinking, consumption of alcoholic beverages, hair dyeing, perineal talc application, as well as for mutual (analgesics-tranquilizers/hypnotics) confounding influences.

TABLE II – DISTRIBUTIONS OF 189 WOMEN WITH OVARIAN CANCER (COMMON EPITHELIAL TUMORS) AND 200 COMPARISON WOMEN BY FREQUENCY OF HAIR DYEING AND PERINEAL TALC APPLICATION. RELATIVE RISK POINT ESTIMATES (95% CONFIDENCE INTERVALS)

	Hair dyeing			*p*-value for trend (1 d.f.)
	Never	≤4 times/year	≥5 times/year	
Cases	112	29	48	
Controls	140	31	29	
RR (crude)	1	1.17 (0.67–2.06)	2.07 (1.23–3.49)	0.008
RR (age-adjusted)	1	1.46 (0.78–2.71)	2.04 (1.17–3.58)	0.014
RR (multiple regression)[1]	1	1.74 (0.91–3.32)	2.16 (1.19–3.89)	0.007
	Talc application in the perineum			*p*-value (1 d.f.)
	No	Yes		
Cases	183	6		
Controls	193	7		
RR (crude)	1	0.90 (0.30–2.74)		0.86
RR (age-adjusted)	1	0.86 (0.27–2.68)		0.78
RR (multiple regression)[1]	1	1.05 (0.28–3.98)		0.95

[1]Controlling for the variables indicated in the footnote to Table I as well as for use of analgesics and tranquilizers/hypnotics and for mutual (hair dyes, talc in the perineal region) confounding influences.

and the respective confidence interval fairly large, there is clearly no evidence of an increased risk associated with perineal application of talc. By contrast, there is a clear, highly significant and dose-dependent positive relation between hair dyeing and risk of ovarian cancer.

DISCUSSION

The strengths and weaknesses of the present study, as a hospital-based case-control investigation, have been considered in another report (Polychronopoulou *et al.*, 1993). Briefly, the study has the power limitations associated with its moderate size and, as in any case-control study, there exists a possibility of selection and, less likely, of information bias. It should be noted, however, that there was no evidence, as contrasted to the theoretical potential, that such biases have actually operated in the present context.

The possibility that ovarian cancer may be caused by exposure to asbestos has been raised by Keal (1960) and elaborated by Graham and Graham (1967). Longo and Young (1979) pointed out that mineral talc is closely related to asbestos, and presented clinical and experimental evidence linking exposure to talc with ovarian cancer. Cramer *et al.* (1982) found a significantly elevated relative risk of 1.92 ($p < 0.003$) among women who reported perineal exposure to talc in comparison to women reporting no such exposure. Since then, 5 other studies have examined the relation between perineal talc application and risk of ovarian cancer, and in none of them was there a statistically significant association (reviewed by Harlow *et al.*, 1992). On the basis of the existing studies, Harlow *et al.* (1992) calculated a significantly elevated weighted relative risk of 1.3 (95% CI 1.1–1.6). The results of the present study do not support an association between talc and ovarian cancer but, given the overlapping range of the confidence intervals, they are not incompatible with it. It should be noted that marginally significant positive associations between talc exposure and risk of ovarian cancer were found in 2 recent case-control studies (Chen *et al.*, 1992; Rosenblatt *et al.*, 1992) that were not included in the meta-analysis by Harlow *et al.* (1992).

Hair coloring products contain components that are mutagenic and carcinogenic in animals (IARC, 1987). An increased risk of breast cancer among long-term users of hair dyes was reported in the 1970's but several subsequent investigations were unable to confirm the early reports (reviewed by Nasca *et al.*, 1992). In contrast, several studies have suggested that occupational exposure to these products may be associated with hematopoietic cancers (reviewed by Zahm *et al.*, 1992) and a recent investigation indicated that users of hair coloring products may be at elevated risk of Hodgkin's disease, non-

Hodgkin's lymphoma, and multiple myeloma (Zahm *et al.*, 1992). The results of the present study suggest that use of hair dyes may increase the risk of ovarian cancer. Although the association in the present study was dose-dependent and statistically significant, and causality is biologically plausible, an etiologic link between hair dyes and ovarian cancer cannot be considered until independent confirmation is available from subsequent studies.

There is no evidence that psychotropic drugs in general or the widely used diazepam in particular is associated with cancer. In an earlier Greek study (Tzonou *et al.*, 1984) a marginally significant positive association was noted between "psychotropic drugs" and ovarian cancer and a similar association was suggested in an earlier report for prochlorperazine and secobarbital (Friedman and Ury, 1980). There was no evidence, in the present study, of an association between tranquilizers and hypnotics, collectively considered, and risk of ovarian cancer. Although diazepam was the most frequently involved compound, the relevant information was not sufficiently reliable to allow drug-specific relative-risk estimates to be made.

Non-steroidal anti-inflammatory drugs, such as aspirin, inhibit the growth of colon tumors in rodents (reviewed by Thun *et al.*, 1991) and there is epidemiologic evidence of a similar effect in humans (Rosenberg *et al.*, 1991; Thun *et al.*, 1991). The mechanism is unknown but may be systemic and involve prostaglandin synthesis or interleukin-6 regulation (Marnett, 1992). In view of this evidence, an inverse association between aspirin and ovarian cancer is not biologically implausible, but a causal interpretation should not be considered before this finding is independently replicated.

The evidence, presented in this report, of an association between ovarian cancer and hair dyes (positive) and analgesics (inverse) is not overwhelming and the respective relations are not very strong. However, the widespread use of hair coloring products and aspirin indicate that if these associations were causal, their population impact would be substantial.

REFERENCES

CHEN, Y., WU, P.C., LANG, J.H., GE, W.J., HARTGE, P. and BRINTON, L.A., Risk factors for epithelial ovarian cancer in Beijing, China. *Int. J. Epidemiol.*, **21**, 23–29 (1992).

CRAMER, D.W., WELCH, W.R., SCULLY, R.E. and WOJCIECHOWSKI, C.A., Ovarian cancer and talc. *Cancer*, **50**, 372–376 (1982).

FRANCESCHI, S., LA VECCHIA, C., BOOTH, M., TZONOU, A., NEGRI, E., PARAZZINI, F., TRICHOPOULOS, D. and BERAL, V., Pooled analysis of 3 European case-control studies of ovarian cancer. II. Age at menarche and at menopause. *Int. J. Cancer*, **49**, 57–60 (1991*a*).

FRANCESCHI, S., PARAZZINI, F., NEGRI, E., BOOTH, M., LA VECCHIA, C., BERAL, V., TZONOU, A. and TRICHOPOULOS, D., Pooled analysis of 3 European case-control studies of ovarian cancer. III. Oral contraceptive use. *Int. J. Cancer*, **49**, 61–65 (1991*b*).

FRIEDMAN, G.D. and URY, H.K., Initial screening for carcinogenicity of commonly used drugs. *J. nat. Cancer Inst.*, **65**, 723–733 (1980).

GRAHAM, J. and GRAHAM, R., Ovarian cancer and asbestos. *Environ. Res.*, **1**, 115–128 (1967).

HANKINSON, S.E., COLDITZ, G.A., HUNTER, D.J., SPENCER, T.L., ROSNER, B. and STAMPFER, M.J., A quantitative assessment of oral contraceptive use and risk of ovarian cancer. *Obstet. Gynecol.*, **80**, 708–714 (1992).

HARLOW, B.L., CRAMER, D.W., BELL, D.A. and WELCH, W.R., Perineal exposure to talc and ovarian cancer risk. *Obstet. Gynecol.*, **80**, 19–26 (1992).

IARC monographs on the evaluation of carcinogenic risks to humans. Suppl. 7. Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1–42. IARC, Lyon (1987).

KEAL, E.E., Asbestosis and abdominal neoplasms. *Lancet*, **ii**, 1211–1216 (1960).

LONGO, D.L. and YOUNG, R.C., Cosmetic talc and ovarian cancer. *Lancet*, **II**, 349–51 (1979).

MARNETT, L.J., Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res.*, **52**, 5575–5589 (1992).

NASCA, P.C., BAPTISE, M.S., FIELD, N.A., METZGER, B.B. and DEMARTINO, R., An epidemiologic case-control study of breast cancer and exposure to hair dyes. *Ann. Epidemiol.*, **2**, 577–586 (1992).

NEGRI, E., FRANCESCHI, S., TZONOU, A., BOOTH, M., LA VECCHIA, C., PARAZZINI, F., BERAL, V., BOYLE, P. and TRICHOPOULOS, D., Pooled analysis of 3 European case-control studies of ovarian cancer. I. Reproductive factors and risk of epithelial ovarian cancer. *Int. J. Cancer*, **49**, 50–56 (1991).

POLYCHRONOPOULOU, A., TZONOU, A., HSIEH, C.C., KAPRINIS, G., REBELAKOS, A., TOUPADAKI, N. and TRICHOPOULOS, D., Reproductive variables, tobacco, ethanol, coffee and somatometry as risk factors for ovarian cancer. *Int. J. Cancer*, **55**, 402–407 (1993).

ROSENBERG, L., PALMER, J.R., ZAUBER, A.G., WARSHAUER, M.E., STOLLEY, P.D. and SHAPIRO, S., A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. *J. nat. Cancer Inst.*, **83**, 355–358 (1991).

ROSENBLATT, K.A., SZKLO, M.S. and ROSENSHEIN, N.B., Mineral fiber exposure and the development of ovarian cancer. *Gynecol. Oncol.*, **45**, 20–25 (1992).

THUN, M.J., NAMBOODIRI, M.M. and HEATH, C.W., Aspirin use and reduced risk of fatal colon cancer. *New Engl. J. Med.*, **325**, 1593–1596 (1991).

TZONOU, A., DAY, N.E., TRICHOPOULOS, D., WALKER, A., SALIARAKI, M., PAPAPOSTOLOU, M. and POLYCHRONOPOULOU, A., The epidemiology of ovarian cancer in Greece: a case-control study. *Europ. J. Cancer clin. Oncol.*, **20**, 1045–1052 (1984).

WHITTEMORE, A.S., HARRIS, R., ITNYRE, J. and THE COLLABORATIVE OVARIAN CANCER GROUP, Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial cancers in white women. *Amer. J. Epidemiol.*, **136**, 1184–1203 (1992*a*).

WHITTEMORE, A.S., HARRIS, R., ITNYRE, J., HALPERN, J. and THE COLLABORATIVE OVARIAN CANCER GROUP, Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. I. Methods. *Amer. J. Epidemiol.*, **136**, 1175–1183 (1992*b*).

WHITTEMORE, A.S., HARRIS, R., ITNYRE, J., HALPERN, J. and THE COLLABORATIVE OVARIAN CANCER GROUP, Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. IV. The pathogenesis of epithelial ovarian cancer. *Amer. J. Epidemiol.*, **136**, 1212–1220 (1992*c*).

ZAHM, S.H., WEISENBURGER, D.D., BABBITT, P.A., SAAL, R.C., VAUGHT, J.B. and BLAIR, A., Use of hair coloring products and the risk of lymphoma, multiple myeloma, and chronic lymphocytic leukemia. *Amer. J. publ. Hlth.*, **82**, 990–997 (1992).

Exhibit 31

Int. J. Cancer: **62,** 678–684 (1995)

Publication of the International Union Against Cancer
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REPRODUCTIVE AND OTHER FACTORS AND RISK OF EPITHELIAL OVARIAN CANCER: AN AUSTRALIAN CASE-CONTROL STUDY

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Of the few factors known to be associated with epithelial ovarian cancer, the most consistently observed relate to women's reproductive function, although even here uncertainties remain. We have undertaken a case-control study involving personal interviews with over 1,600 women, the largest of its kind to date, to investigate further the associations between women's reproductive histories and other factors and the development of ovarian cancer. Cases were drawn from women diagnosed with epithelial ovarian cancer in 3 Australian states, Queensland, New South Wales and Victoria, between August 1990 and December 1993, and controls were drawn at random from the electoral roll, stratified by age and geographic region. Trained interviewers administered standard questionnaires to obtain detailed information about women's reproductive and contraceptive histories and other factors of interest, such as smoking and family history of ovarian or other cancer. Findings were based on data from 824 cases and 860 controls and confirmed the reduced risk of ovarian cancer associated with increasing parity and duration of use of the oral contraceptive pill (OCP), hysterectomy and tubal ligation. The strongest association of all was seen with use of the OCP for 10 years or more. An inverse association between ovarian cancer and age at first birth was observed, but this was not statistically significant. There were no associations between development of ovarian cancer and number of incomplete pregnancies, use of hormone replacement therapy or menstrual history. Among other factors considered, education after leaving school was negatively associated and high body mass index, family history of ovarian cancer, use of talc in the abdominal or perineal region and smoking were positively associated with occurrence of ovarian cancer.
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Ovarian cancer, the 6th most common cancer occurring in women, accounts for 4% of all incident cancers and 5% of all cancer deaths among women (Silverberg *et al.,* 1990). Its incidence varies between different countries and among different ethnic groups, ranging from 4 to 15 per 100,000 women per year (age-standardised to the world population; Parkin *et al.,* 1992). In Australia, the age-standardised incidence rate for ovarian cancer is approximately 9 per 100,000 (Jelfs *et al.,* 1992).

Several factors have been reported as being associated with the risk of epithelial ovarian cancer, most notably various reproductive and menstrual factors. Compared with nulliparous women, parous women have a lower risk of developing ovarian cancer (Whittemore *et al.,* 1992; Kelsey and Hildreth, 1983), with an estimated 15% reduction in risk associated with each birth or pregnancy (Whittemore *et al.,* 1992; Booth *et al.,* 1989; Hartge *et al.,* 1989). Late age at first live birth has been positively associated with ovarian cancer in some (Polychronopoulou *et al.,* 1993; Booth *et al.,* 1989) but not all studies (Cramer *et al.,* 1983; Kelsey and Hildreth, 1983), while in a recent Swedish study, after adjustment for parity and age, risk of disease decreased with later ages at first birth (Adami *et al.,* 1994). Miscarriages and terminated pregnancies, termed "incomplete pregnancies" hereafter, may reduce risk to the same degree as full-term pregnancies (Booth *et al.,* 1989; Kvåle *et al.,* 1988), although again other results are not supportive (Polychronopoulou *et al.,* 1993; Hartge *et al.,* 1989). After adjustment for parity, breast-feeding has been reported to reduce risk of disease by up to 40%, but this reduction did not appear to be related to duration of breast-feeding (Whittemore *et al.,* 1992; Booth *et al.,* 1989) and was entirely absent in some instances (Hartge *et al.,* 1989; Cramer *et al.,* 1983).

With regard to menstrual factors, a moderately increased risk (20%) has been seen in women with early menarche in some settings (Whittemore *et al.,* 1992; Franceschi *et al.,* 1991; Kelsey and Hildreth, 1983). Some researchers have noted significant associations between late age of menopause and increased risk of disease (Franceschi *et al.,* 1991; Booth *et al.,* 1989), while others found no support for this relationship (Kvåle *et al.,* 1988). No significant links between ovarian cancer and menorrhagia, dysmenorrhoea or duration of menstrual flow have been recorded (Kelsey and Hildreth, 1983).

Of particular interest with respect to the aetiology of ovarian cancer, because it is a discretionary exposure, is the apparent protective role of the oral contractive pill (OCP). A recent review examined 21 studies with data on the association between OCP use and subsequent ovarian cancer risk. After adjustment for parity, women who had ever used the OCP had a lower risk of epithelial ovarian cancer of around 40% compared with women who had never used the OCP (Parazzini *et al.,* 1991). The protective effect of OCP use on ovarian cancer risk increases with duration of use, with up to 70% reduction seen for more than 5 years of use compared with around 5% reduction for less than 1 year of use (Whittemore *et al.,* 1992). Similarly, consistent inverse associations have been found between ovarian cancer and certain gynaecological procedures. Tubal sterilisation has been associated with a 50% reduction in risk of ovarian cancer, and a 20–40% reduction has also been seen following hysterectomy with ovarian conservation (Whittemore *et al.,* 1992; Booth *et al.,* 1989).

Regarding other possible risk factors, a history of any cancer among first-degree relatives has been shown to be associated with an increase in ovarian cancer, estimated to be around 80% (Cramer *et al.,* 1983). In many, but not all studies, ovarian cancer patients belonged to a higher socio-economic class than controls (Booth *et al.,* 1989; Kelsey and Hildreth, 1983), and risk of disease has also been found to decrease with increasing years of education (Polychronopoulou *et al.,* 1993). While a slight increase in risk has been associated with perineal exposure to talc (Chen *et al.,* 1992), most studies have not found a statistically significant association (Parazzini *et al.,*

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1991). In a review of existing studies, Harlow *et al.* (1992) calculated a significant (30%) elevation in relative risk associated with perineal talc use.

We present here data from a large case-control study of epithelial ovarian cancer to examine these issues further, with a view to resolving some of the uncertainties.

SUBJECTS AND METHODS

All histologically confirmed incident cases of primary epithelial ovarian cancer diagnosed between January 1991 and December 1992 and registered in all major gynaecological–oncology treatment centres in the 3 most populous Australian states, New South Wales, Victoria and Queensland, were ascertained. From Queensland, an unselected sample of 30 histologically confirmed cases diagnosed between 1 August, 1990 and 31 December, 1990 were also included, as well as all cases diagnosed in 1993. The histopathology for each case was reviewed by an independent pathologist in each state (P.R., B.S., G.W.) by examining tissue used to establish the original diagnosis. When the consent of each woman's treating doctor had been obtained, patients were invited to participate either prior to discharge after initial surgery or when attending the hospital clinic for subsequent follow-up. Cases, aged between 18 and 79 years, with whom contact could be made and who were capable of completing the questionnaire were eligible for inclusion in the study.

Controls were chosen from the electoral roll by a random procedure designed to yield an age distribution similar to that anticipated among cases (enrolment to vote is compulsory in Australia). For each case a control listed as being from a geographic region similar in degree of urbanisation was selected and a letter explaining the study and inviting participation was sent to the address listed on the electoral register. If a woman was not found at the registered address, every attempt was made to trace her current whereabouts. Women who were unable to be located and invited to participate, those incapable of completing the questionnaire and those who gave a history of ovarian cancer or bilateral oophorectomy were not eligible to take part.

Identically trained interviewers administered a standard questionnaire in a face-to-face interview either in the clinic (cases) or in the subject's home (some cases and all controls). Questions were asked about marital status, education, ethnicity, height and weight, smoking history, occupation of self and partner and family history of ovarian and any other cancers. Details of each woman's reproductive and contraceptive histories were obtained by means of a pregnancy and a contraceptive calendar which elicited, month by month, events in the woman's reproductive life from ages 15 to 50 years. Questions were also asked about difficulties in conceiving, abdominal surgery and other factors, such as history of talc use or childhood mumps infection. In addition, dietary data were collected from all participants using a self-completed food frequency questionnaire adapted from the instrument developed and validated by Willett *et al.* (1985).

A number of demographic, environmental, reproductive, hormonal and biological factors were examined for possible associations with ovarian cancer. Crude odds ratios (ORs) with confidence limits were calculated as estimates of the relative risk of ovarian cancer. After stratifying by parity, the Mantel-Haenszel estimate for combining stratum-specific ORs was used to calculate adjusted ORs. Relative risks were estimated using multiple categorical logistic regression to simultaneously adjust for parity, hysterectomy, tubal ligation and duration of OCP use and also for possible confounders such as age (in years), education, talc use, body mass index (BMI), smoking and family history of cancer. In addition, menstrual factors, age at first birth, multiple pregnancies, breast-feeding, incomplete pregnancies and pregnancy difficulties were entered separately in the above base model. Analyses which explored associations with breast-feeding, multiple births (twins, etc.) and age at first birth were confined to parous women. All analyses were performed using the SAS statistical package.

RESULTS

Of a total of 1,116 cases of epithelial ovarian cancer identified, 201 (18%) were ineligible. There were 26 cases excluded because their tumour was deemed to be metastatic rather than of ovarian origin, and 12 tumours were excluded on histopathological review of tissue sections, with 4 reclassified as non-epithelial, 7 as benign, and 1 as recurrent ovarian cancer. A further 82 women were found to be outside the eligible age range; 68 women were unable to complete the questionnaire due to language difficulties or psychiatric conditions (n = 49) or because they were too ill (n = 19); and 12 women were unable to be contacted to invite participation. A further case (with peritoneal tumour) was excluded because of a previous bilateral oophorectomy. Of the 915 eligible cases, 824 (90%) were interviewed. With regard to the eligible cases not interviewed, 50 had died before an interview could take place, 34 (4%) refused and in 7 cases the doctor did not grant permission for interview. Among 1,527 women listed on the electoral roll who were randomly selected to take part as controls, 192 could not be traced, 105 were unable to complete the questionnaire due to language or psychiatric problems (n = 69) or because of sickness or death (n = 36), 48 were found to have had a previous bilateral oophorectomy and 4 were out of the nominated age range. Of the remaining 1,178 eligible controls, 860 (73%) agreed to be interviewed. Considering the histopathological categories of cases in the study, 22 (3%) had cancer of primary peritoneal origin (*i.e.,* extra-ovarian serous carcinoma) and 140 (17%) had borderline epithelial ovarian tumours, leaving 665 (80%) with frankly malignant tumours. The age distributions of cases and controls were similar (Table I); the mean age of cases was 55.5 years and of controls, 54.8 years.

Education beyond school was crudely associated with a 23% decrease in risk of ovarian cancer and a 31% decrease after adjustment for parity (Table I). Women with a BMI greater than the 85th percentile had approximately double the risk of ovarian cancer compared with women in the middle 30% of the BMI range (taken as normal weight), after adjustment for parity. Among nulliparous women there was a 41% increase in risk for women who had ever been married or lived with a partner compared with those who had never lived with a partner. Women who said they used talc in the abdominal or perineal regions had a small increase (27%) in risk of ovarian cancer, and a small increase (17%) was observed for ever-smokers compared to life-long non-smokers (Table I). After adjustment for duration of OCP use, there was a significant positive relation between smoking and ovarian cancer (OR = 1.38, 95% CI: 1.12–1.69; data not shown).

One of the strongest associations observed was between ovarian cancer and immediate family history of the disease. Women with a first-degree relative previously diagnosed with ovarian cancer had an approximately 4-fold increase in risk of disease compared with women who had no immediate family history of disease. An immediate family history of any cancer other than ovarian cancer did not appear to have a significant effect on the risk of disease (Table I).

Of the gynaecological factors assessed, hysterectomy, tubal ligation and any OCP use were all inversely related to ovarian cancer (Table II). Long duration of OCP use was strongly and inversely related to disease, with a 70% reduction in risk seen for 10 or more years of use compared with never use. Hormone replacement therapy (HRT) also was inversely, albeit not

TABLE I – DISTRIBUTION OF VARIOUS PERSONAL AND SOCIAL FACTORS AMONG CASES AND CONTROLS. CRUDE ODDS RATIOS (WITH 95% CONFIDENCE LIMITS) AND ODDS RATIOS ADJUSTED FOR PARITY

Factor	Cases (n = 824)[1] (%)	Controls (n = 860) (%)	Crude odds ratio	Odds ratio adjusted for parity
Age (yr)				
18–29	4.2	5.5	0.79 (0.50–1.27)	0.56 (0.31–1.00)
30–39	9.2	8.1	1.15 (0.79–1.67)	1.02 (0.68–1.53)
40–49	19.4	23.8	0.83 (0.62–1.09)	0.82 (0.61–1.10)
50–59	25.8	19.5	1.34 (1.02–1.77)	1.40 (1.05–1.86)
60–69	25.2	25.6	1.0	1.0
70–79	16.0	17.4	0.93 (0.69–1.26)	0.88 (0.65–1.20)
Postschool education	(n = 818)	(n = 855)		
Yes	42.4	48.8	0.77 (0.64–0.94)	0.69 (0.57–0.84)
BMI	(n = 805)	(n = 852)		
< 15th percentile	14.0	15.6	1.10 (0.81–1.49)	1.00 (0.73–1.37)
15–35th percentile	19.6	20.3	1.18 (0.89–1.56)	1.14 (0.86–1.51)
35–65th percentile	27.0	32.9	1.0	1.0
65–85th percentile	21.1	19.3	1.34 (1.01–1.77)	1.39 (1.04–1.84)
≥ 85th percentile	18.3	12.0	1.86 (1.37–2.53)	1.97 (1.44–2.70)
Married or lived with a partner	92.0	93.5	0.80 (0.55–1.16)	1.41 (0.93–2.13)[2]
Ovarian cancer in mother, sister or daughter	2.7	0.7	3.90 (1.58–9.68)	4.48 (1.96–10.3)
Family history of any cancer other than ovarian cancer	41.9	41.0	1.03 (0.85–1.26)	1.08 (0.88–1.31)
Use of talc around abdomen/ perineum	56.7	52.0	1.21 (1.00–1.46)	1.27 (1.04–1.54)
Smoking				
Current or ex-smoker	41.9	37.8	1.19 (0.98–1.44)	1.17 (0.96–1.43)
Life-long non-smoker	58.1	62.2	1.0	1.0

[1]Total is 824 cases and 860 controls unless otherwise specified.–[2]Adjusted by restricting to nulliparous women.

TABLE II – DISTRIBUTION OF CERTAIN GYNAECOLOGICAL FACTORS AMONG CASES AND CONTROLS. CRUDE ODDS RATIOS (WITH 95% CONFIDENCE LIMITS) AND ODDS RATIOS ADJUSTED FOR PARITY

Factor	Cases (n = 824)[1] (%)	Controls (n = 860) (%)	Crude OR	OR adjusted for parity
Hysterectomy (with ovarian conservation)	14.1	20.1	0.65 (0.50–0.84)	0.71 (0.55–0.92)
Tubal ligation	12.6	22.7	0.49 (0.38–0.64)	0.56 (0.43–0.73)
Infection in tubes or uterus	(n = 817)	(n = 852)		
Yes	6.5	6.7	0.97 (0.66–1.42)	0.91 (0.62–1.35)
Ever used OCP	50.5	64.5	0.56 (0.46–0.68)	0.58 (0.48–0.71)
Length of use of OCP	(n = 797)	(n = 845)		
Never used	51.2	36.1	1.0	1.0
< 24 months	14.8	15.6	0.67 (0.50–0.89)	0.70 (0.53–0.94)
24–59 months	11.9	11.6	0.72 (0.53–1.00)	0.74 (0.53–1.02)
60–119 months	13.8	16.9	0.58 (0.43–0.77)	0.59 (0.44–0.79)
≥ 120 months	8.3	19.8	0.29 (0.21–0.41)	0.29 (0.21–0.40)
Ever used HRT	24.2	26.5	0.88 (0.71–1.10)	0.93 (0.74–1.16)

[1]Total is 824 cases and 860 controls unless otherwise specified.

significantly, associated with ovarian cancer (Table II), as was a reported history of infection in the tubes or uterus.

Regarding relationships between reproductive factors and ovarian cancer, women of parity 2 or more had a significantly lower risk of disease than nulliparous women (Table III). Multiple pregnancies appeared to decrease risk of ovarian cancer (not significantly) after restricting analysis to parous women and adjusting for parity. Breast-feeding appeared to be unrelated to risk of ovarian cancer after adjustment for parity. Although on crude analysis age at first birth was not associated with risk of disease, after adjustment for parity there was an inverse trend with later ages at first birth (Table III) ($\chi^2_{trend} = 3.79, p = 0.05$).

Menstrual factors, including age at menarche, menopausal status and age at menopause, were not significantly related to the occurrence of ovarian cancer (Table IV). Irregular menstrual cycles were weakly associated with disease, and there appeared to be a decreased risk associated with light menstrual flow and an increase associated with heavy menstrual flow, though again none of these was significant. Women who had experienced painful periods were found to have a slightly increased risk compared with women who never had painful periods.

On multivariate analysis, the trend of increasing risk of ovarian cancer with increasing height-adjusted weight calculated from the lowest (below the 15th percentile) to the highest (equal to or greater than the 85th percentile) categories remained highly significant. Hysterectomy and tubal ligation were shown to be independently associated with a decreased risk of ovarian cancer, as was ever use of OCP (Table V). A strong inverse trend was observed with increased duration of OCP use: 10 or more years of use compared to never use was associated with a 74% reduction in risk of disease (Table V). An inverse relation was also seen in women with 2 or more live births, with risk decreasing with increasing number of births. Furthermore, late age at first birth was inversely related to

TABLE III – DISTRIBUTION OF VARIOUS REPRODUCTIVE FACTORS AMONG CASES AND CONTROLS. CRUDE ODDS RATIOS (WITH 95% CONFIDENCE LIMITS) AND ODDS RATIOS ADJUSTED FOR PARITY

Factor	Cases (n = 824)[1] (%)	Controls (n = 860) (%)	Crude OR	OR adjusted for parity
Parity				
0	22.7	15.6	1.0	
1	14.0	7.4	1.29 (0.88–1.88)	
2	28.8	31.1	0.64 (0.48–0.84)	
3	17.8	25.2	0.48 (0.36–0.66)	
4	9.2	13.4	0.47 (0.33–0.68)	
5+	7.5	7.3	0.70 (0.47–1.07)	
Age at first birth (yr)[2]	(n = 640)	(n = 727)		
15–19	10.6	8.9	1.22 (0.84–1.77)	1.13 (0.77–1.66)
20–24	45.9	47.0	1.0	1.0
25–29	30.5	31.4	0.99 (0.78–1.27)	0.91 (0.71–1.18)
30–34	9.2	8.8	1.07 (0.73–1.58)	0.81 (0.54–1.22)
≥35	3.8	3.9	1.00 (0.57–1.76)	0.64 (0.34–1.20)
Multiple pregnancies (twins, etc.)	1.6	3.0	0.51 (0.26–1.00) 0.56 (0.29–1.09)[2]	0.62 (0.32–1.21)[2]
Incomplete pregnancies				
0	71.2	69.7	1.0	1.0
1	19.5	20.1	0.95 (0.74–1.21)	1.04 (0.81–1.33)
2	4.6	5.6	0.81 (0.52–1.26)	0.89 (0.57–1.38)
3+	4.6	4.6	0.97 (0.61–1.53)	0.94 (0.59–1.49)
Unsuccessfully tried to become pregnant	12.5	7.3	1.81 (1.30–2.52)	1.34 (0.94–1.90)
Ever breast-fed	61.9	69.6	0.71 (0.58–0.87) 0.85 (0.65–1.12)[2]	0.96 (0.73–1.28)[2]

[1]Total is 824 cases and 860 controls unless otherwise specified.–[2]Restricted to parous women.

TABLE IV – DISTRIBUTION OF VARIOUS MENSTRUAL FACTORS AMONG CASES AND CONTROLS. CRUDE ODDS RATIOS (WITH 95% CONFIDENCE LIMITS) AND ODDS RATIOS ADJUSTED FOR PARITY

Factor	Cases (n = 824)[1] (%)	Controls (n = 860) (%)	Crude OR	OR adjusted for parity
Age at menarche (yr)	(n = 822)	(n = 859)		
<12	14.6	15.5	0.95 (0.72–1.26)	0.95 (0.71–1.27)
12–13	45.9	46.2	1.0	1.0
≥14	39.5	38.3	1.04 (0.84–1.28)	1.07 (0.86–1.32)
Menopausal status				
Pre-menopausal	39.1	39.4	1.0	1.0
Post-menopausal	60.9	60.6	1.01 (0.83–1.23)	1.09 (0.89–1.33)
Age at menopause (yr)	(n = 498)	(n = 517)		
<45	10.0	8.9	1.22 (0.78–1.89)	1.19 (0.76–1.86)
45–49	26.9	24.6	1.14 (0.84–1.54)	1.07 (0.79–1.46)
50–52	43.8	47.3	1.0	1.0
≥53	20.3	19.2	1.14 (0.82–1.59)	1.11 (0.79–1.56)
Regularity of periods	(n = 820)	(n = 858)		
Very regular	78.7	76.3	1.0	1.0
Sometimes irregular	13.2	14.3	0.89 (0.67–1.18)	0.87 (0.66–1.15)
Very irregular	8.2	9.3	0.85 (0.60–1.20)	0.89 (0.63–1.25)
Menstrual flow	(n = 818)	(n = 857)		
Light	6.0	8.0	0.76 (0.52–1.12)	0.72 (0.49–1.05)
Average	59.3	60.7	1.0	1.0
Heavy	34.7	31.3	1.14 (0.92–1.40)	1.11 (0.90–1.37)
Painful periods	(n = 820)	(n = 856)		
Often	27.1	26.9	1.17 (0.91–1.50)	1.14 (0.88–1.47)
Sometimes	26.8	22.8	1.36 (1.05–1.77)	1.32 (1.02–1.72)
Seldom	17.7	17.4	1.18 (0.88–1.57)	1.19 (0.89–1.58)
Never	28.4	32.9	1.0	1.0

[1]Total is 824 cases and 860 controls unless otherwise specified.

occurrence of ovarian cancer, though not significantly. There was no association with disease in relation to either number of incomplete pregnancies overall (Table V) or number of miscarriages or number of terminated pregnancies taken separately.

Painful periods appeared to be positively associated with disease after adjustment for other factors, and there was a moderate linear relationship observed between menstrual flow and risk of disease (Table V). Breast-feeding, age at menopause and age at menarche were not significantly associated with disease. Women who had ever had a multiple pregnancy were at decreased risk, but the confidence intervals were wide (Table V).

DISCUSSION

The size of our case-control study of epithelial ovarian cancer has enabled us to confirm some of the known associations and report some significant associations not previously

TABLE V – ADJUSTED ODDS RATIOS (WITH 95% CONFIDENCE LIMITS) FOR REPRODUCTIVE AND MENSTRUAL VARIABLES, HYSTERECTOMY, TUBAL LIGATION, PARITY, DURATION OF OCP USE AND BMI, ALSO ADJUSTING FOR AGE, POSTSCHOOL EDUCATION, FAMILY HISTORY OF CANCER (OTHER THAN OVARY), TALC USE ON ABDOMEN OR GENITALS AND SMOKING STATUS

Factors	Adjusted OR (95% ci)	χ^2 for trend (p value)
BMI (percentile)		
< 15th	1.03 (0.74–1.44)	
15–35th	1.26 (0.93–1.70)	
35–65th	1.0	
65–85th	1.50 (1.12–2.03)	
≥ 85th	2.00 (1.44–2.79)	12.30 ($p < 0.001$)
Hysterectomy	0.69 (0.52–0.91)	
Tubal ligation	0.60 (0.45–0.80)	
Ever used OCP[1]	0.54 (0.43–0.70)	
Length of use of OCP		
Never used	1.0	
< 24 months	0.65 (0.47–0.90)	
24–59 months	0.69 (0.48–1.01)	
60–119 months	0.53 (0.37–0.75)	
≥ 120 months	0.26 (0.18–0.38)	47.43 ($p < 0.001$)
Ever used HRT	1.03 (0.80–1.33)	
Parity		
0	1.0	
1	1.38 (0.92–2.08)	
2	0.82 (0.59–1.13)	
3	0.61 (0.43–0.86)	
4	0.52 (0.35–0.78)	
5+	0.84 (0.53–1.33)	11.90 ($p < 0.001$)
Age at first birth[2] (yr)		
15–19	0.96 (0.62–1.47)	
20–24	1.0	
25–29	0.93 (0.71–1.22)	
30–34	0.87 (0.56–1.36)	
≥ 35	0.61 (0.32–1.15)	1.43 ($p = 0.23$)
Multiple pregnancies[2]	0.68 (0.33–1.38)	
Incomplete pregnancies		
0	1.0	
1	1.05 (0.81–1.37)	
2	0.90 (0.56–1.46)	
3+	1.02 (0.61–1.70)	0.00 ($p = 0.99$)
Unsuccessfully tried to become pregnant	1.21 (0.83–1.77)	
Ever breast-fed[2]	1.05 (0.77–1.44)	
Age at menarche (yr)		
< 12	0.87 (0.69–1.10)	
12–13	1.0	
≥ 14	1.07 (0.85–1.35)	1.52 ($p = 0.22$)
Menopausal status		
Pre-menopausal	1.0	
Post-menopausal	0.72 (0.50–1.03)	
Age at menopause (yr)		
< 45	1.04 (0.65–1.68)	
45–49	1.05 (0.74–1.50)	
50–52	1.0	
≥ 53	1.14 (0.78–1.66)	0.08 ($p = 0.78$)
Menstrual flow		
Light	0.71 (0.47–1.08)	
Average	1.0	
Heavy	1.10 (0.87–1.39)	3.00 ($p = 0.08$)
Painful periods		
Often	1.23 (0.93–1.62)	
Sometimes	1.32 (0.99–1.74)	
Seldom	1.30 (0.95–1.78)	
Never	1.0	2.33 ($p = 0.13$)

[1]Duration of OCP use not in the model.–[2]Restricted to parity ≥ 1.

observed. Use of the OCP, after adjustment for other factors, appeared to reduce the risk of ovarian cancer by 46% compared to never use. Moreover, risk decreased the longer the use of OCP: women who took the pill for at least 10 years had an apparent 74% reduction in risk compared with never users. Tubal ligation and hysterectomy with conservation of at least one ovary each reduced the risk of disease by 30–40%. These apparent protective effects of OCP use, tubal ligation and hysterectomy with ovarian conservation are consistent with previous findings (Whitemore *et al.,* 1992; Booth *et al.,* 1989). Our results indicate a strong and significant trend of decreasing risk of epithelial ovarian cancer with each live birth after the first, as has been seen by others (Parazzini *et al.,* 1991; Booth *et al.,* 1989). The slight up-turn with 5 or more births may reflect chance, as indicated by the width of the confidence interval. Early age at first birth, after adjustment for parity, has previously been reported as being protective against ovarian cancer, with an elevated risk when age at first birth was 35 years or more (Parazzini *et al.,* 1991). In contrast, we have found that risk of epithelial ovarian cancer decreased with increasing age at first live birth after adjusting for parity and duration of OCP use (not statistically significant). This is consistent with the results of a Swedish study (Adami *et al.,* 1994), which were adjusted for parity but not for other possible confounders such as OCP use. Pooled analyses of 6 population-based case-control studies (Whittemore *et al.,* 1992) also showed significant inverse associations with late age at first birth.

No obvious effects of age at menarche or age at menopause were observed, which is in accordance with a number of studies (Kvåle *et al.,* 1992; Whittemore *et al.,* 1992). Taken together, the evidence from these would seem to outweigh the positive relationships with early age at menarche and late age of menopause found in some studies (Franceschi *et al.,* 1991; Booth *et al.,* 1989). A positive association with painful periods has not been found consistently by others (Kelsey and Hildreth, 1983) and may reflect either chance or differential recall. Some have found a protective effect of incomplete pregnancies after adjusting for parity (Booth *et al.,* 1989; Parazzini *et al.,* 1991); we found no reduction in risk. Consistent with prior work, no significant associations were found between lactation (Hartge *et al.,* 1989; Cramer *et al.,* 1983) or HRT (Wu *et al.,* 1988) and risk of ovarian cancer. The strong positive trend ($p < 0.001$) noted with increasing BMI, such that women in the highest 15th percentile had a 2-fold increase in risk compared with those of normal height-adjusted weight, has been observed in at least 2 previous studies (Cramer *et al.,* 1984; Farrow *et al.,* 1989).

Immediate family history of ovarian cancer was a strong predictor of epithelial ovarian cancer, supporting the view that a significant genetic component exists in at least the small percentage of ovarian cancer cases so affected (Cramer *et al.,* 1983). Some other associations were observed here that have not been consistently observed in smaller case-control studies. Education beyond secondary school after adjusting for other factors, using multivariate logistic analysis, was associated with a significant reduction in risk (OR = 0.77, CI 0.62–0.95), as has been observed before (Polychronopoulou *et al.,* 1993), suggesting that socio-economic status needs further examination together with other environmental factors that could explain this relationship. Regular use of talc in the region of the abdomen or perineum was associated with a slight increase in risk of disease, which, though not observed in some studies (Parazzini *et al.,* 1991), supports other findings (Booth *et al.,* 1989; Harlow *et al.,* 1992) and other clinical and experimental evidence linking exposure to talc with ovarian cancer (Longo and Young, 1979). Perhaps of particular note is that cancer of the ovary is not considered to be tobacco-related (Polychronopoulou *et al.,* 1993). In this study, however, ever being a smoker, after adjustment for duration of OCP use, appeared to significantly increase risk of disease by almost 40%, and we intend to explore this association in greater detail in future analyses.

In considering possible biases firstly with respect to the selection of controls ascertained from the electoral roll, the

electoral roll status of all cases was investigated. Of the 824 cases, 750 (91%) were enrolled, 28 (3%) had never been on the electoral roll and for 46 cases (6%) electoral roll status could not be determined. Analyses were repeated, including only cases who were on the electoral roll, with no material alteration in the results. Given the control participation rate of 73% it is also possible that in some instances the exposure patterns among study controls differed systematically from those of the underlying source population of Australian women. The age distribution of non-responders considered in 3 age bands (18–39, 40–59 and 60–79 years) and according to state of residence was virtually identical to that of participating controls, and thus selection bias due to a systematic age difference between responders and non-responders seems unlikely. Turning to the case subjects, the patient populations were considered to be generally representative of all cases occurring in women living in the states of Queensland (based on checks against cases of epithelial ovarian cancer registered in the Queensland Cancer Registry showing that more than 95% of cases had been captured) and Victoria because these are comparatively small states and medical services are relatively centralised (though the precise capture rate is unknown). In the largest and most populous state of New South Wales, however, identified cases represented around half the cases of ovarian cancer diagnosed in the period (probably with under-representation of women in rural areas). However, stratification by state gave similar patterns of risk, suggesting that the potential selection bias in ascertaining the New South Wales cases, and perhaps the Victorian cases to some extent, did not materially distort the findings. Finally, during analysis it was assumed that borderline and frankly malignant tumours have a similar aetiology. In support of this assumption, no differences in risk estimates between the 2 groups were found when cases with borderline and frankly malignant tumours were analysed separately.

Recall and interviewer bias should have been minimised because of the highly structured and standardised questionnaire delivered by identically trained interviewers and by the use of detailed calendars for complex contraceptive and pregnancy histories. Although all data presented here were self-reported, other studies have shown that data relating to menstrual history, reproductive history and operations are reasonably valid (Bean *et al.,* 1979). Further, adjustment was made for all known confounders in the analysis, although, of course, confounding by unknown factors is always possible.

The theory that incessant ovulation is a factor in the development of ovarian cancer, first developed by Fathalla (1971), is supported by some, but not all, evidence from epidemiological studies. Reductions in risk associated with pregnancy and OCP use are the main factors that support the hypothesis as ovulation ceases during these times. In the present study we conclusively found that childbirth and OCP use were associated with reduced risk of disease and that risk decreased with increased duration of OCP use and increased parity, which support the hypothesis. Other protective factors, such as hysterectomy and tubal ligation, do not appear relevant to the incessant ovulation theory as ovulation would not normally be suppressed after these surgeries. The hypothesis would also predict a reduced risk with late age at menarche and early age at menopause, which has not been shown here. The increase in risk associated with dysmenorrhoea after adjustment for OCP use might be taken to offer indirect support to the incessant ovulation theory as ovulation is believed to be a pre-requisite for menstrual cramps in the following cycle. An alternative theory is that high gonadotrophin levels increase the risk of disease (Cramer and Welch, 1983) and that suppression of gonadotrophin secretion would reduce risk of ovarian cancer, which is again the case during OCP use and pregnancy. Obesity would be expected to decrease risk under this hypothesis if gonadotrophin concentrations are reduced in these women; however, a significant positive relationship has been seen between obesity and risk of disease here and elsewhere (Farrow *et al.,* 1989; Cramer *et al.,* 1984).

Among reproductive characteristics not considered in detail here in relation to ovarian cancer, low fertility remains a factor to be fully investigated, including the quantification of ovulatory history as far as possible. Women of low parity should be categorised into those choosing not to have children and those who have had difficulties in conceiving or bearing children, to precisely determine the effects of sub-fertility on risk of epithelial ovarian cancer. We plan to address these issues in subsequent publications.

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REFERENCES

ADAMI, H., HSIEH, C., LAMBE, M., TRICHOPOULOS, D., LEON, D. and PERSSON, I., Parity, age at first childbirth, and the risk of ovarian cancer. *Lancet,* **344,** 1250–1254 (1994).

BEAN, J.A., LEEPER, J.D., WALLACE, R.B., SHERMAN, B.M. and JAGGER, H., Variations in the reporting of menstrual histories. *Amer. J. Epidemiol.,* **109,** 181–185 (1979).

BOOTH, M., BERAL, V. and SMITH, P., Risk factors for ovarian cancer: a case-control study. *Brit. J. Cancer,* **60,** 592–598 (1989).

CHEN, Y., WU, P.C., LANG, J.H., GE, W.J., HARTGE, P. and BRINTON, L.A., Risk factors for epithelial ovarian cancer in Beijing, China. *Int. J. Epidemiol.,* **21,** 23–29 (1992).

CRAMER, D.W., HUTCHINSON, G.B., WELCH, W.R., SCULLY, R.E. and RYAN, K.J., Determinants of ovarian cancer risk. I. Reproductive experiences and family history. *J. nat. Cancer Inst.,* **71,** 711–716 (1983).

CRAMER, D.W. and WELCH, W.R., Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. *J. nat. Cancer Inst.,* **71,** 717–721 (1983).

CRAMER, D.W., WELCH, W.R., HUTCHINSON, G.B., WHILLETT, W. and SCULLY, R.E., Dietary animal fat in relation to ovarian cancer risk. *Obstet. Gynecol.,* **63,** 833–838 (1984).

FARROW, D.C., WEISS, N.S., LYON, J.L. and DALING, J.R., Association of obesity and ovarian cancer in a case-control study. *Amer. J. Epidemiol.,* **129,** 1300–1304 (1989).

FATHALLA, M.F., Incessant ovulation—a factor in ovarian neoplasia? *Lancet,* **2,** 163 (1971).

FRANCESCHI, S., LA VECCHIA, C., BOOTH, M., TZONOU, A., NEGRI, E., PARAZZINI, F., TRICHOPOULOS, D. and BERAL, V., Pooled analysis of 3 European case-control studies of ovarian cancer: II. Age at menarche and at menopause. *Int. J. Cancer,* **49,** 57–60 (1991).

HARLOW, B.L., CRAMER, D.W., BELL, D.A. and WELCH, W.R., Perineal exposure to talc and ovarian cancer risk. *Obstet. Gynecol.,* **80,** 19–26 (1992).

HARTGE, P., SCHIFFMAN, M.H., HOOVER, R., MCGOWAN, L., LESHER, L. and NORRIS, H.J., A case-control study of epithelial ovarian cancer. *Amer. J. Obstet. Gynecol.,* **161,** 10–16 (1989).

JELFS, P., GILES, G., SHUG, D., TAYLOR, R., BONETT, A., THOMPSON, J., RING, I. and DURLING, G., *Cancer in Australia 1983–1985.* Cancer Series **1,** Australian Institute of Health and Welfare, p. 127, Australian Government Publishing Service, Canberra (1992).

KELSEY, J.L. and HILDRETH, H.G., *Breast cancer and gynecologic cancer epidemiology,* pp. 94–115, CRC Press, Boca Raten, FL (1983).

KVÅLE, G., HEUCH, I. and NILSSEN, S., Is the risk of ovarian cancer

related to age at menarche and age at menopause? *Int. J. Cancer,* **51,** 333–334 (1992).

KVÅLE, G., HEUCH, I., NILSSEN, S. and BERAL, V., Reproductive factors and risk of ovarian cancer: a prospective study. *Int. J. Cancer,* **42,** 246–251 (1988).

LONGO, D.L. and YOUNG, R.C., Cosmetic talc and ovarian cancer. *Lancet,* **2,** 349–351 (1979).

PARAZZINI, F., FRANCESCHI, S., LA VECCHIA, C. and FASOLI, M., The epidemiology of ovarian cancer—review. *Gynecol. Oncol.,* **43,** 9–23 (1991).

PARKIN, D.M., MUIR, C.D., WHELAN, S.L., GAO, Y.-T., FERLAY, J. and POWELL, J. (eds.), *Cancer incidence in five continents, vol.* **VI,** pp. 966–967, IARC Scientific Publications **120,** Lyon (1992).

POLYCHRONOPOULOU, A., TZONOU, A., HSIEH, C., KAPRINIS, G., REBELAKOS, A., TOUPADAKI, N. and TRICHOPOULOS, D., Reproductive variables, tobacco, ethanol, coffee and somatometry as risk factors for ovarian cancer. *Int. J. Cancer,* **55,** 402–407 (1993).

SILVERBERG, E., BORING, C.C. and SQUIRES, T.S., Cancer statistics. *Cancer J. Clin.* **40,** 9–26 (1990).

WHITTEMORE, A.S., HARRIS, R., ITNYRE, J. and THE COLLABORATIVE OVARIAN CANCER GROUP, Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies II. Invasive epithelial ovarian cancers in white women. *Amer. J. Epidemiol.,* **136,** 1184–1203 (1992).

WILLETT, W.C., SAMPSON, L., STAMPFER, M.J., ROSNER, B., BAIN, C., WITSCHI, J., HENNEKENS, C.H. and SPEIZER, F.E., Reproducibility and validity of a semiquantitative food frequency questionnaire, *Amer. J. Epidemiol.,* **122,** 51–65 (1985).

WU, M.L., WHITTEMORE, A.S., PAFFENGAGER, R.S., SARLES, D.L., KAMPERT, J.B., GROSSER, S., JUNG, D.L., BALLON, S., HENDRICKSON, M. and MOHLE-BOETANI, J., Personal and environmental characteristics related to epithelial ovarian cancer. I Reproductive and menstrual events and oral contraceptive use. *Amer. J. Epidemiol.,* **128,** 1216–1227 (1988).

APPENDIX

Survey of Women's Health Study Research Group

Collaborators: Sèamus Campbell, Chris Dalrymple, Arthur Day, Alan Ferrier, Keith Free[1], Peter Grant, Paul Harnett, Phil Harvey, Roger Houghton, Tom Jobling, Peter MacCullum Cancer Institute, Robert Planner, Tony Proietto, Robert Rome, John Solomon, Gerry Wain.

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[1]Deceased.

Exhibit 32

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Human menopausal gonadotropin and the risk of epithelial ovarian cancer*

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Objective: To determine whether women with epithelial ovarian cancer are more likely to have been exposed to fertility drugs, and in particular hMG, than healthy population controls.
Design: A nationwide case-control study.
Patients: Two hundred living women 36 to 64 years of age, with a histologically confirmed diagnosis of primary invasive or borderline epithelial ovarian cancer that was first diagnosed and reported to the Israel Cancer Registry between January 1, 1990 and September 1, 1993 were enrolled. There were 164 (82%) invasive and 36 (18%) borderline epithelial ovarian tumors among the 200 cases. The controls were 408 women from the same dialing areas selected by random digit dialing. Cases and controls were interviewed using a standard questionnaire. A multivariate logistic model was used to assess the association of fertility drug use and ovarian cancer, controlling for variables found to be statistically associated with this outcome on univariate analysis.
Results: Twenty-four women with epithelial ovarian cancer (12%) and 29 healthy controls (7.1%) reported that they had used any fertility drug (adjusted odds ratio [OR] 1.31; 95% confidence interval [CI] 0.63 to 2.74). Among cases and controls, respectively, 22 and 24 reported that they had used hMG alone or in combination with clomiphene citrate (adjusted OR 1.42, 95% CI 0.65 to 3.12), and 11 and 6 reported that they had used hMG alone (adjusted OR 3.19, 95% CI 0.86 to 11.82). The risk was increased particularly in the subgroup of women with borderline ovarian tumors who had used hMG (adjusted OR 9.38, 95% CI 1.66 to 52.08).
Conclusions: We conclude that the use of ovulation induction agents, in particular hMG, may increase the risk of epithelial ovarian tumors. Fertil Steril 1996;65:13–8

Key Words: Ovulation induction, gonadotropin, infertility, ovarian cancer, borderline ovarian tumors

During the last five years investigators have raised considerable concern about the potential of increased risk of ovarian cancer associated with drugs administered for induction of ovulation (1). A variety of case reports of women exposed to these drugs who subsequently developed ovarian cancers have been published (2–4). Concern has been heightened further as a result of two recently published studies (5, 6). The first study, a combined analysis of data from 12 case-control studies, reported that, among nulligravid women, fertility drugs were associated with an increased risk of invasive epithelial (5), borderline epithelial (7), and non-epithelial ovarian tumors (8). The second study, a

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case-cohort study (6), reported that after prolonged use of clomiphene citrate (CC) the risk of invasive and borderline ovarian tumors was elevated among both gravid and nulligravid women. The relative risks associated with the use of other drugs that induce ovulation were not estimated due to the low prevalence of exposure to other agents among that cohort.

These clinical observations, taken together with theoretical considerations such as Fathalla's (9) "incessant ovulation" hypothesis, necessitate further evaluation of this question. This is especially important in view of the increasing use of modern techniques for assisted reproduction that involve potent follicle stimulation regimens. We performed a nationwide case-control study to determine whether women with epithelial ovarian cancer in general, and borderline epithelial tumors in particular, are more likely to have been exposed to fertility drugs than healthy population controls.

MATERIALS AND METHODS

Since 1982 notification of malignant and related diseases to the Israel Cancer Registry by physicians and hospitals has been compulsory by law (completeness is considered to be approximately 95%). Cases of ovarian cancer were identified from this Registry. Cases were eligible for this study if they had a histologically confirmed diagnosis of primary infiltrating or borderline epithelial ovarian cancer that was first diagnosed and reported between January 1, 1990 and September 1, 1993; if they were born between January 1, 1929 and December 31, 1957 (fertility drugs were first used in Israel in 1960, therefore, this age group would have had an opportunity for exposure); and if they were alive at the time of the interview. Living cases only were used so that ascertainment of exposure could be based on personal interviews exclusively (25% of women with ovarian cancer reported to the Cancer Registry between the above dates had died before the study period). Of 287 living women who satisfied our case definition, we interviewed 200 (70%). The others were not interviewed because of inability to locate the patient or physician (25%), illness (1%), refusal by the physician (1%), or refusal by the patient (3%).

There were 164 (82%) invasive and 36 (18%) borderline epithelial ovarian tumors among the 200 cases. Among living cases not interviewed, the ratio of invasive to borderline ovarian tumors was identical to that of the cases interviewed. The distribution of histologic diagnoses is shown in Table 1.

Controls were obtained by telephoning randomly selected numbers within the same area codes as those of the cases, a method closely resembling that reported by Hartge and colleagues (10), and were interviewed during the same period as the cases. Thus, cases and controls were matched for geographic area by the sampling procedure. Eligibility for controls was based on date of birth in the identical range to that of the cases. Once a household was reached, the interviewer asked if a woman born between January 1, 1929 and December 31, 1957 resided there. Women who had undergone bilateral oophorectomy (1%) were excluded as controls. Two controls were selected for each case. To obtain the final sample of 408 controls, 4,983 calls were made, resulting in 2,072 households reached. Of these households, 16.1% had no women, 26.3% had no women of the appropriate date of birth, 9.8% had women of the appropriate date of birth but who were not at home, 16.2% refused or were too busy to be interviewed, and 10.7% spoke no Hebrew.

Two trained interviewers, a physician (A.S.) and a medical student, conducted all interviews between November 28, 1993 and August 6, 1994. Cases and controls were interviewed using a standard questionnaire designed to collect information similarly for the two groups. Cases were asked to relate all answers to the period before their date of diagnosis. The questionnaire contained detailed items of obstetric and gynecologic history, including extensive information on infertility and its treatment. For technical reasons the physician (A.S.) interviewed more cases than controls, whereas the opposite was true of the latter interviewer. Ten percent of the cases and controls were selected randomly for reinterview by the alternate interviewer using a shortened questionnaire for interexaminer reliability. The results of this interobserver check showed Kappa values of 0.7 to 1.0 for categorical variables and correlation coefficients of 0.99 to 1.0 for continuous variables. In addition, 12 women who reported use of fertility drugs but could not remember the

Table 1 *Histologic Diagnosis in the Women With Epithelial Ovarian Cancer**

Histologic diagnosis	No. of women (n = 200)
Invasive (n = 164)	
Adenocarcinoma	21 (10.5)
Serous	90 (45.0)
Mucinous cystadenocarcinoma	11 (5.5)
Endometrioid carcinoma	24 (12.0)
Clear cell adenocarcinoma	3 (1.5)
Mixed epithelial	3 (1.5)
Other	12 (6.0)
Borderline (n = 36)	
Serous cystadenocarcinoma	30 (15.0)
Mucinous cystadenocarcinoma	6 (3.0)

* Values are cases with percentages in parentheses.

type were reinterviewed. All questionnaires were checked for consistency and accuracy of coding.

The study was designed to detect an odds ratio (OR) of 3.0 for the use of fertility drugs, given an α level of 0.05 and power of 90%, a 2:1 ratio of controls to cases, and based on a presumed exposure rate to fertility drugs among the controls of 5%. The data were analyzed using SPSS for the PC (SPSS Inc., Chicago, IL). Differences in sociodemographic characteristics, habits, and gynecologic history were assessed using χ^2 with Yates' correction for categorical variables and t-test for continuous variables. The association between ovarian cancer and fertility drug use was measured using the OR. The only matching variable was the telephone area code. A conditional logistic model revealed that this matching did not alter the magnitude, direction, or significance of the reported associations with fertility drugs. Therefore a nonconditional multivariate logistic model was used to assess the association of fertility drug use and ovarian cancer controlling for variables found to be associated statistically with this outcome on univariate analysis. Confidence intervals (CI) on ORs were calculated using the method of Woolf (11). Because of the imbalance of cases and controls interviewed, a variable representing interviewer was included in the logistic model. In assessing the association between fertility drugs and ovarian cancer, comparisons were made between those reporting use of specific drugs and nonusers of any drug.

The study protocol was submitted and approved by the institutional review board of Hadassah Medical Organization and the Ministry of Health. For legal reasons, women ascertained via the Cancer Registry could not be contacted directly. Rather, their physicians were contacted and consent to contact the patient was obtained through them. Verbal consent was obtained from both cases and controls, and subsequent written confirmation of this consent was obtained from all subjects except controls who chose to remain anonymous.

RESULTS

As compared with the controls, the women with ovarian cancer tended to be better educated, to come from European-American background, to be unmarried, and were more likely to report a family history of ovarian cancer (Table 2). There were no significant differences between ovarian cancer cases and controls in coffee consumption, smoking, or vitamin use, but a larger proportion of cases than controls reported using talc. Cases had a mean body mass index (BMI) of 25.91 whereas the mean BMI was 25.05 in controls ($P = 0.02$). The mean age at diagnosis or interview was 50.0 years for cases and 49.9 years for controls. In addition, cases were more likely to be nulligravida, nulliparous, to have lower parity, to report secondary infertility (data not shown), and less likely to have breast-fed (Table 2). However, in contrast to previous large case-control studies (12), we did not find a significant negative association between oral contraceptive use and epithelial ovarian cancer for either borderline or malignant tumors. Seventeen percent of cases and 11.3% of controls ($P = 0.07$) reported that they had undergone an infertility work-up, but most subjects could not recall or identify the particular cause of infertility.

Twenty-four women with epithelial ovarian cancer (12%) and 29 healthy controls (7.1%) reported that they had used any fertility drug (Table 3). Twelve women (2 cases and 10 controls) who on first interview did not recall the type of drug used were reinterviewed. After reinterview, six (all controls) recalled that the fertility drug was CC. One control reported that she had used corticosteroids and five women (two cases and three controls) remained who could not recall the type of fertility drug used. These women were excluded from the analyses regarding specific drugs. On final analysis, among cases and controls, respectively, 11 and 18 reported that they

Table 2 Sociodemographic, Obstetric, and Gynecologic Characteristics of Cases and Controls

	No. of women*		
	Cases (n = 200)	Controls (n = 408)	P value
Age at diagnosis (y)			
36 to 40	27 (13.5)	49 (12.0)	
41 to 49	66 (33.0)	173 (42.4)	
50 to 59	75 (37.5)	109 (26.7)	
60 to 64	32 (16.0)	77 (18.9)	0.025
Region of birth			
Europe-America	105 (52.5)	118 (28.9)	
Israel	57 (28.5)	183 (44.9)	
Asia-Africa	38 (19.0)	107 (26.2)	<0.0001
Education >12 years	101 (50.5)	150 (36.8)	0.001
Marital status, ever married	184 (92.0)	398 (97.5)	0.001
Ovarian cancer in mother or sister	22 (11.0)	24 (5.9)	0.04
Smoking, ever	141 (70.5)	271 (66.4)	0.35
Talc			
Never-seldom	178 (89.0)	385 (94.4)	
Moderate-a lot	21 (10.5)	23 (5.6)	0.04
Mean age at menarch (y)	12.78	13.1	0.01
Nulligravida	16 (8.0)	14 (3.4)	0.025
Parity			
Nulliparous	34 (17.0)	21 (5.1)	
1 to 3	127 (63.5)	238 (58.3)	
≥4	39 (19.5)	149 (36.5)	<0.0001
Breastfeeding, never	73 (36.5)	96 (23.5)	0.001
Oral contraceptive usage, ever	42 (21.0)	97 (23.8)	0.51
Investigated for infertility	34 (17.0)	46 (11.3)	0.07

* Values in parentheses are percentages.

Table 3 Odds Ratio of Ovarian Epithelial Tumors Associated With Use of Fertility Drugs

	All epithelial tumors				Borderline tumors		
Drug	Cases*	Controls*	Crude odds ratio	Adjusted odds ratio†	Cases*	Crude odds ratio	Adjusted odds ratio†
Any‡	24 (12.0)	29 (7.1)	1.78 (0.97 to 3.27)	1.31 (0.63 to 2.74)	10 (27.7)	5.03 (2.04 to 12.22)	3.52 (1.23 to 10.09)
CC only	11 (5.9)	18 (4.5)	1.32 (0.57 to 3.01)	0.88 (0.33 to 2.34)	2 (5.5)	1.62 (0.25 to 7.87)	1.28 (0.25 to 6.87)
hMG	11 (5.9)	6 (1.6)	3.95 (1.33 to 12.2)	3.19 (0.86 to 11.82)	6 (16.7)	14.58 (3.82 to 55.91)	9.38 (1.66 to 52.08)
hMG and/or CC	22 (11.1)	24 (6.0)	1.97 (1.03 to 3.77)	1.42 (0.65 to 3.12)	8 (22.2)	4.86 (1.81 to 12.79)	3.08 (0.98 to 9.69)

* Values are number of women with percentages in parentheses.

† Adjusted OR is adjusted for age, parity, BMI, region of birth, education, family history, and interviewer. Values in parentheses are 95% CI.

‡ Comparing users and nonusers of any fertility drug.

had used CC only, 22 and 24 reported that they had used CC alone or in combination with hMG, and 11 and 6 reported that they had used hMG. One woman reported she had used only hCG and another reported treatment with corticosteroids.

In the univariate analyses (Table 3), women with epithelial ovarian cancer were more likely to have been exposed to fertility drugs than healthy population controls (OR 1.78, 95% CI 0.97 to 3.27). A logistic regression model including age, education, region of birth, parity, family history of ovarian cancer, BMI, and interviewer resulted in an adjusted OR of 1.31 (95% CI 0.63 to 2.74). No association was noted between use of CC and ovarian cancer in the univariate or multivariate analyses (Table 3). Only 10 subjects (4 cases and 6 controls) reported that they had used CC for ≥12 cycles (crude OR 1.44, 95% CI 0.34 to 5.82). Multivariate adjustment was not performed due to the small numbers.

Compared with untreated women, women who reported ever having used hMG, in any combination with other drugs and for any period, had a higher risk of having epithelial ovarian cancer (crude OR 3.95, 95% CI 1.33 to 12.02). Adjusting for the variables noted above resulted in an OR of 3.19 (95% CI 0.86 to 11.82). Women with borderline tumors were significantly more likely to have been exposed to ovulation induction agents (adjusted OR 3.52, 95% CI 1.23 to 10.09) and particularly to hMG (adjusted OR 9.38, 95% CI 1.66 to 52.08). This association was not demonstrated when invasive tumors were considered alone (data not shown).

DISCUSSION

This study shows that use of ovulation induction agents is associated with ovarian cancer. Specifically, these results indicate that epithelial ovarian tumors were roughly three times as likely to develop in women who had used hMG compared with women who did not use this agent. Furthermore, we have demonstrated a very strong association between this agent and borderline ovarian tumors (adjusted OR 9.38, 95% CI 1.66 to 52.08).

Our study had a number of limitations, many of which relate to the case-control design. We had no access to the medical records of subjects, thus we could not verify the information about exposure to fertility drugs that was obtained from the study participants. Recall bias cannot be excluded; if cases were more likely than controls to report the use of fertility drugs, the magnitude of the positive association may have been overestimated. More controls than cases could not recall the type of infertility drug used; however, on reinterview, no further use of hMG was elicited. We elicited more controls who had used CC, further influencing the results toward the null hypothesis. Another cause for concern is that most subjects could not recall the particular cause of infertility, thus stratification according to different types of infertility, and specifically ovulatory problems, could not be performed. We cannot exclude the possibility that our results were confounded by a specific cause of infertility.

The selection of cases for this study was based only on vital status and ability to locate the subject. Nevertheless, exclusion of women who had died already may limit the generalizability of our findings in that a positive association may be relevant only for less severe cases of ovarian cancer. This is supported by the increased strength of association found for women with borderline tumors. Other possible sources of selection bias must be explored as well. Although in the present study cases and controls differed significantly with respect to country of origin and other baseline characteristics, controls were representative of the Israeli population of this age group (13), whereas cases exhibited most of the established risk factors for ovarian cancer including low parity, positive family history, higher education, lower age at menarche, and higher talc use. We ad-

justed for these risk factors (except for talc use and age at menarche because they did not contribute significantly to the model) in the logistic model.

On the other hand, the plausibility of these results is heightened by biologic theory, epidemiologic findings, and other recent studies that have suggested that fertility drugs might have neoplastic effects (5–8). Of particular interest has been Fathalla's (9) theory that "extravagant" and incessant ovulation in women may be an inciting factor for the development of epithelial ovarian cancer. According to this hypothesis, each ovulation causes minor trauma to the surface epithelium of the ovary, and the recurrent trauma increases the risk for cancerous changes within this epithelium. Epidemiologic studies have supported this theory (12, 14). Factors that promote ovulation such as fertility drugs may thus increase the risk of ovarian cancer.

Our findings generally are consistent with two recent investigations of the association fertility drugs use and ovarian cancer. The first study, a meta-analysis of data from 12 case-control studies reported that among nulligravid women, fertility drugs were associated with an increased risk of epithelial and nonepithelial ovarian tumors (5, 7, 8). However, in that study, no information was provided on the specific fertility drugs used, which has led to understandable skepticism whether women in those studies were exposed to "new" fertility drugs such as CC and hMG that were registered in the United States after 1967 (15). Many investigators therefore have believed that further evaluation is warranted (15–18). Indeed, Rossing et al. (6) recently reported the results of a case-cohort study that aimed to determine whether infertile women have an increased risk of ovarian tumors and, if so, whether that risk is influenced by the apparent cause of the infertility or its treatment. The authors concluded that, after prolonged use of CC (>12 cycles), the risk of invasive and borderline ovarian tumors was elevated among both gravid and nulligravid women (relative risk 11.1, 95% CI 1.5 to 82.3). The relative risks associated with the use of other drugs that induce ovulation were not estimated due to the low prevalence of exposure to other agents among that cohort.

Unlike Rossing and colleagues (6) we did not find an association between epithelial ovarian cancer and prolonged exposure to CC. Rather, we found that women with epithelial ovarian tumors were exposed more often to induction of ovulation by hMG. A possible explanation for these differences might be that in our study only a very small group of women was exposed to ≥12 cycles of CC treatment (four cases). Most physicians in Israel would treat a woman with CC for three to six cycles and, if she does not become pregnant, would switch the treatment to hMG. Thus, the subgroup of cases treated by hMG is characterized by failure of previous treatment with CC and, therefore, it is the subgroup of infertile women who use fertility drugs for a longer period. Our results therefore may be in concordance with those reported by Rossing et al. (6), suggesting that the length of treatment with ovulation induction agents, rather than the drug per se, may constitute a major risk factor.

The OR among women with borderline tumors was much higher than that among women with invasive cancer (Table 3). This finding too is consistent with those demonstrated in previous studies (6) and clinical data (2–4, 19). A possible explanation of this finding might be that ovarian stimulation induces highly differentiated indolent tumors. The natural history of borderline tumors still is unknown and it is not clear whether borderline tumors, if undetected, develop into invasive tumors. Thus, until further investigation clarifies whether there are indeed etiologic differences between these two histologic categories of ovarian tumors, it is difficult to exclude the possibility of ascertainment bias. The ovaries of women treated by fertility drugs are screened more frequently than those of nonusers, and such screening of women might lead to more frequent ascertainment of borderline tumors.

The suggestion that prolonged use of ovulation induction agents is associated with ovarian tumors is of particular importance in light of the increasing popularity of fertility and assisted reproduction clinics. In 1988, in the United States, approximately 2 million women reported previous exposure to fertility drugs (Chandra A, Krulewitch CJ, Hoffman HJ, abstract). Furthermore, whereas in the past these drugs were given only to women with anovulatory infertility, today the tendency is to treat even normally-ovulating women to induce superovulation. Although at present there is insufficient evidence to change treatment policies, we believe that in view of these concerns it might be advisable to provide women with this relevant information and obtain informed consent before ovulation induction is administered. We propose that until more data are collected, women who use fertility drugs be under regular gynecologic surveillance.

In conclusion, our findings suggest that use of ovulation induction agents, in particular hMG, may increase the risk of epithelial ovarian tumors. The strong association observed with hMG treatment in particular may be due to the prolonged nature of exposure to fertility drugs of these women. However, because of the limitations of the case-control design, further investigation is necessary.

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REFERENCES

1. Fishel S, Jackson P. Follicular stimulation for high tech pregnancies: are we playing it safe? Br Med J 1989;299:309–311.
2. Atlas M, Mencjer J. Massive hyperstimulation and borderline malignancy carcinoma of the ovary: a possible association. Acta Obstet Gynecol Scand 1982;61:261–3.
3. Goldberg GL, Runowicz CD. Ovarian carcinoma of low malignant potential, infertility, and induction of ovulation—Is there a link? Am J Obstet Gynecol 1992;166:853–4.
4. Shushan A, Brzezinski A, Schenker JG. Hormonal treatments and genital cancer: an update. In: Genazzani AR, Petraglia F, Genazzani AD, editors. Frontiers in gynecologic and obstetric investigation. New York: The Parthenon Publishing Group LTD, 1993:163–84.
5. Whittemore AS, Harris R, Itnyre J, the Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Am J Epidemiol 1992;136:1184–203.
6. Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG. Ovarian tumors in a cohort of infertile women. N Engl J Med 1994;331:771–6.
7. Harris R, Whittemore AS, Itnyre J, the Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. III. Epithelial tumors of low malignant potential in white women. Am J Epidemiol 1992;136:1204–11.
8. Horn-Ross PL, Whittemore AS, Itnyre J, the Collaborative Ovarian Cancer Group. Characteristics relating ovarian cancer risk: collaborative analysis of 12 US case-control studies. VI. Nonepithelial cancers among adults. Epidemiology 1992;3:490–5.
9. Fathalla MF. Incessant ovulation—a factor in ovarian neoplasia? Lancet 1971;2:163.
10. Hartge P, Brinton LA, Rosenthal JF, Cahill JI, Hoover RN, Waksberg J. Random digit dialing in selecting a population-based control group. Am J Epidemiol 1984;120:825–33.
11. Schlesselman JJ. Case-control studies. Design, conduct, analysis. New York: Oxford University Press, 1982.
12. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. The reduction in risk of ovarian cancer associated with oral-contraceptive use. N Engl J Med 1987;316:650–5.
13. Central Bureau of Statistics. Statistical abstract of Israel 1994;45:94–5.
14. McGowan L, Parent L, Lednar W, Norris HJ. The woman at risk for developing ovarian cancer. Gynecol Oncol 1979; 7:325–44.
15. International Federation of Fertility Societies (IFFS). Fertility drugs and ovarian cancer. Fertil Steril 1993;60:406–8.
16. Walters DE. Ovarian cancer and pregnancy: comment on a paper by Whittemore et al. Fertil Steril 1994;61:239–42.
17. Spirtas R, Kaufman SC, Alexander NJ. Fertility drugs and ovarian cancer: red alert or red herring? Fertil Steril 1993;59:291–3.
18. Dietl J. Ovulation and ovarian cancer. Lancet 1991;338:445.
19. Nijman HW, Burger CW, Baak JP, Schats R, Vermorken JB, Kenemans P. Borderline malignancy of the ovary and controlled hyperstimulation, a report of 2 cases. Eur J Cancer 1992;28A:1971–3.

Exhibit 33

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TUBAL STERILISATION, HYSTERECTOMY AND DECREASED RISK OF OVARIAN CANCER

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We have examined the effect of tubal sterilisation and hysterectomy on risk of ovarian cancer in a large case-control study in eastern Australia involving 824 women aged 18–79 years, diagnosed with epithelial ovarian cancer between 1990 and 1993, and 855 controls randomly selected from the electoral roll. Relative risks for ovarian cancer were estimated using multiple categorical regression to adjust for age, parity, oral contraceptive use and other risk factors. Tubal sterilisation was associated with a 39% reduction in risk of ovarian cancer (RR 0.61, 95% CI 0.46–0.85) and hysterectomy with a 36% reduction (RR 0.64, 95% CI 0.48–0.85). Risk remained low 25 years after surgery and was reduced irrespective of sterilisation technique, and estimates were similar among various types of epithelial ovarian cancer. The greatest reduction (74%) was observed among women with primary peritoneal tumours. Pelvic infection and use of vaginal sprays or contraceptive foams were not related to ovarian cancer, while use of talc in the perineal region slightly but significantly increased risk among women with patent fallopian tubes. Reportedly heavy or painful menses, perhaps associated with retrograde flow, were associated with ovarian cancer, and reduction in risk of disease after hysterectomy was greatest among women who had heavy periods. Our findings support the theory that contaminants from the vagina, such as talc, and from the uterus, such as endometrium, gain access to the peritoneal cavity through patent fallopian tubes and may enhance the malignant transformation of ovarian surface epithelium. Surgical tubal occlusion may reduce the risk of ovarian cancer by preventing the access of such agents. *Int. J. Cancer* 71:948–951, 1997.
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Although the factors that cause epithelial ovarian cancer are unknown, there are several discretionary factors that appear to protect against it. Oral contraception is associated with up to 70% reduction in risk after 10 or more years of use compared with never-use (Purdie *et al.,* 1995), the protection presumably reflecting long-term suppression of ovulation. A 30–50% reduction in risk has been observed (Booth *et al.,* 1989; Hankinson *et al.,* 1993; Irwin *et al.,* 1991; Whittemore *et al.,* 1992), though not consistently (Chen *et al.,* 1992; Risch *et al.,* 1994; Shu *et al.,* 1989), after tubal sterilisation, and this is independent of childbearing and oral-contraceptive use. A similar inverse association is found between hysterectomy with ovarian conservation and ovarian cancer (Hankinson *et al.,* 1993; Hartge *et al.,* 1989; Irwin *et al.,* 1991; Risch *et al.,* 1994; Weiss and Harlow, 1986).

Explanations include blocking the ascent into the peritoneal cavity (Woodruff, 1971) of carcinogenic agents such as talc (Henderson *et al.,* 1979), asbestos (Graham and Graham, 1967), viruses (Wahlberg, 1994) or contraceptive foams or gels (Silver, 1994) through surgical closure of the fallopian tubes or post-surgical compromise of ovarian circulation (Cattanach, 1985) associated with decreased ovarian function. Alternatively, the negative associations between pelvic surgery and ovarian cancer may be secondary to sub-fertility (Mori *et al.,* 1992) or may be the result of surveillance bias since women whose ovaries have been screened for malignancy during surgery will have a reduced risk of cancer for several years compared with women not screened in this manner (Weiss and Harlow, 1986). Available data are largely inconclusive about these alternatives since the studies have involved small numbers of women reporting tubal sterilisation and hysterectomy and details regarding timing of surgery often were lacking. In the largest case-control study of its kind, we have studied in greater detail the possible effect of tubal sterilisation or hysterectomy on a woman's risk of developing epithelial ovarian cancer, specifically investigating most of the associated factors that have been postulated to date.

SUBJECTS AND METHODS

Incident cases of primary epithelial ovarian cancer diagnosed between August 1990 and December 1993 and registered in gynaecological-oncology treatment centres in 3 Australian states, New South Wales, Victoria and Queensland, were ascertained. Tissue used to establish original diagnoses was reviewed by an independent pathologist in each state. Full details have been presented elsewhere (Purdie *et al.,* 1995). Briefly, cases aged 18–79 years were invited to participate in the study with their doctors' consent, and a response rate of 90% was obtained. Control women, frequency-matched for age and urban/rural district of residence, were randomly chosen from the electoral roll (enrolment to vote is compulsory in Australia), and a letter explaining the study and inviting participation was sent to them. Women who gave a history of ovarian cancer or bilateral oophorectomy were excluded, and the response rate was 73% among eligible controls. In a face-to-face interview, identically trained interviewers administered a standard questionnaire, asking about personal details such as education, height and weight, smoking history, details of menstrual cycles and family history of ovarian cancer. Full histories of pregnancies and lactation were obtained, and by means of a calendar, each woman's contraceptive practices between the ages of 15 and 50 years were elicited. Questions also were asked about history of pelvic infection, abdominal surgery and use of talc. If tubal sterilisation or hysterectomy had been performed, women were asked about date and place of surgery and name of surgeon. Confirmation and details

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of operative procedures were then sought from the relevant medical practitioners with each woman's written consent.

To test the theory that tubal occlusion prevents entry of foreign agents to the peritoneal cavity through the fallopian tubes, we assessed exposures to vaginal sprays, contraceptive foams and douches, possible talc lubricant on the surface of condoms (Kasper and Chandler, 1995) and talc used specifically in the perineal region. It also was postulated that if peritoneal irritants were to play a role in the development of epithelial ovarian cancers, then this would apply to primary peritoneal cancers in particular, with a consequent substantial reduction in risk to this sub-group after tubal occlusion. Duration of exposure was calculated from age at first use to earliest age at pelvic surgery, if any, and age at last use (age at diagnosis or at interview if use was continuing). In addition, we investigated whether tubal occlusion might prevent retrograde menstruation, which may be damaging to the ovary. To this end, associations between ovarian cancer and painful or heavy periods were assessed on the assumption that these symptoms identified women who may have experienced retrograde menstruation (Smith, 1991) to a greater degree than women without these symptoms.

Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated as estimates of the relative risk (RR) of ovarian cancer. Multivariate RRs were estimated using multiple categorical logistic regression to simultaneously adjust for parity and duration of oral contraceptive use and for other possible confounders, such as age (in years), education, body mass index, smoking and history of ovarian cancer in a first-degree relative. Multivariate RRs are presented in this report except when stated otherwise. All analyses were performed using the SAS (Cary, NC) statistical package.

RESULTS

Tubal sterilisation

Of 824 women with incident ovarian cancer and 855 controls, there were 104 cases (13%) and 194 controls (23%) who reported tubal sterilisation. Among a random sample of 64 women for whom surgical records could be located, there was 100% agreement with the women's self-reports of tubal sterilisation (Green *et al.*, 1997). Among control subjects, women who had had a tubal sterilisation tended to have had more children more often than those who had not had tubal sterilisation (Table I). Risk of ovarian cancer was appreciably reduced in women after tubal sterilisation compared with other women (RR 0.61, 95% CI 0.46–0.85) (Table II). Among a random sample of 20 cases and 58 controls for whom information about method of tubal sterilisation was available from either surgical records or women's general practitioners, decreased risk of ovarian cancer was observed irrespective of sterilisation technique, with crude RRs of 0.15, 95% CIs 0.02–1.3 after occlusion by tubal rings; 0.20, 0.04–0.95 after bipolar diathermy; 0.23, 0.07–0.84 after application of clips; and 0.48, 0.24–0.94 after tubal ligation.

TABLE I – PREVALENCE OF POSSIBLE RISK FACTORS AMONG CONTROLS WITH AND WITHOUT SURGICAL OCCLUSION OF THE FALLOPIAN TUBES, STANDARDIZED TO THE AGE DISTRIBUTION OF ALL CONTROL SUBJECTS[1]

Risk factor	With tubal sterilisation (n = 194)	Without tubal sterilisation (n = 661)	With hysterectomy (n = 171)	Without hysterectomy (n = 684)
Post-school education	41.4	49.8	47.5	48.3
Ever smoked	48.1	37.1	34.3	39.0
Parity ≥2	94.9	74.3	86.0	76.5
Heavy periods	34.8	31.1	51.7	26.1
Painful periods	61.0	48.5	63.2	46.1
Ever used oral contraceptives	68.2	63.0	63.8	64.7
Past history of pelvic infection	8.3	6.0	8.5	6.1
Ever used talc in perineal region	41.1	41.1	38.9	40.3

[1]Values are percentages.

Risk remained low 25 years or more after tubal sterilisation, when there was a 57% reduction in risk of ovarian cancer (Table II).

Hysterectomy

There were 116 cases, and 178 of 860 initially enlisted controls who reported undergoing hysterectomy with conservation of at least one ovary prior to the date of index diagnosis. Subsequent checks of available medical records revealed that bilateral oophorectomy had been performed in 5 controls, thereafter excluded from all analyses; and in a validation study (Green *et al.*, 1997), it was shown that 2 cases and 2 controls had not had a previous hysterectomy, leaving 114 cases (14%) and 171 controls (20%) for study. Controls with a previous hysterectomy were more likely to have had more children or heavy, painful periods than those without (Table I). Estimated risk of ovarian cancer among women after hysterectomy was reduced compared with women without such a history (RR 0.64, 95% CI 0.48–0.85), with maximum effect reached 15 or more years after surgery (Table II).

Surgical tubal occlusion

Among women who had had occlusion of the fallopian tubes through either or both of these surgical procedures, there was, predictably, a 37% reduction in risk of ovarian cancer compared with women who had had neither procedure (RR 0.63, 95% CI 0.49–0.79). There was little material variation in the results among the main histological sub-types of ovarian cancer or between borderline and frankly malignant tumours. After surgical tubal occlusion, the risk of developing a serous tumour, the largest histological sub-group, was reduced by 46% (RR 0.54, 95% CI 0.42–0.70), and the sub-group showing the greatest reduction in risk was that of women who had primary peritoneal tumours (RR 0.26, 95% CI 0.07–0.87).

Pelvic infection before surgery was not related to risk of epithelial ovarian cancer, and neither was duration of use of vaginal sprays or of contraceptive foams related to risk. Ever-douching for contraceptive purposes was associated with a non-significant 60% increase in risk of ovarian cancer. A modest association was seen between ovarian cancer and use of talc in the perineal region (RR 1.3, 95% CI 1.1–1.6). There was no additional effect of longer duration of talc use nor was there any relation to reported age when talc was first used in the perineal region. No associations with duration of partner's use of condoms (which may have had talc lubricants) or with duration of use of a diaphragm (which may have been stored in talc) were evident. Compared with women who had neither used talc nor had surgical sterilisation, risk was highest among talc users without surgery (RR 1.3, 95% CI 1.0–1.7) and lowest among women with a history of tubal sterilisation or hysterectomy who had not applied talc to the perineum (RR 0.6, 95% CI 0.50–0.84).

Habitual heavy periods and painful periods were each weakly associated with ovarian cancer (RR 1.2, 95% CI 0.93–1.4 and RR 1.1, 95% CI 0.86–1.4, respectively), and risk of epithelial ovarian cancer among women with either heavy or painful periods was raised to a similar level (RR 1.2, 95% CI 1.0–1.5) overall and for the main histological sub-groups. Women who reported heavy periods showed a 20% larger reduction in risk of ovarian cancer after hysterectomy (RR 0.54) than women who had light or normal menstrual loss (RR 0.74), though the reduction in risk after tubal sterilisation was similar whether or not women reported heavy periods. Women who had experienced painful periods had a lower risk of ovarian cancer after tubal sterilisation or after hysterectomy compared with women who reported pain-free periods (Table III).

DISCUSSION

Ovarian cancer was found to be significantly reduced by 39% after tubal sterilisation and by 36% after hysterectomy. Women who had these procedures tended to have had more children than other women and, thus, were already at lower risk of ovarian cancer, but the low risk persisted after adjustment for parity and other factors, which is consistent with previous findings (Booth *et*

TABLE II – DISTRIBUTION OF CASES AND CONTROLS ACCORDING TO TIME BETWEEN TUBAL STERILISATION AND HYSTERECTOMY AND DATE OF DIAGNOSIS AMONG CASES, AND RISK OF OVARIAN CANCER ADJUSTED FOR AGE, EDUCATION, BODY MASS INDEX, PARITY, DURATION OF ORAL-CONTRACEPTIVE USE, SMOKING AND FAMILY HISTORY OF OVARIAN CANCER

Time since surgery (years)	Tubal sterilisation					Hysterectomy				
	Cases		Controls		Relative risk (95% confidence interval)	Cases		Controls		Relative risk (95% confidence interval)
	Number	(%)	Number	(%)		Number	(%)	Number	(%)	
No surgery	720	(87)	661	(77)	1.0	708	(86)	684	(80)	1.0
Ever surgery	104	(13)	194	(23)	0.61 (0.46–0.85)	114	(14)	171	(20)	0.64 (0.48–0.85)
0–4	9	(1)	25	(3)	0.42 (0.19–0.96)	15	(2)	18	(2)	1.5 (0.73–3.3)
5–9	14	(2)	28	(3)	0.56 (0.27–1.1)	18	(2)	23	(3)	0.89 (0.45–1.7)
10–14	29	(4)	48	(6)	0.72 (0.43–1.2)	22	(3)	33	(4)	0.67 (0.37–1.2)
15–19	36	(4)	47	(6)	0.98 (0.60–1.6)	19	(2)	37	(4)	0.52 (0.28–0.94)
20–24	8	(1)	28	(3)	0.26 (0.11–0.62)	17	(2)	29	(3)	0.54 (0.28–1.1)
25+	8	(1)	18	(2)	0.43 (0.18–1.0)	25	(3)	31	(4)	0.49 (0.28–0.89)

TABLE III – TUBAL STERILISATION, HYSTERECTOMY AND RISK OF OVARIAN CANCER IN RELATION TO MENSTRUAL HISTORY: RELATIVE RISKS (95% CONFIDENCE INTERVALS) ADJUSTED FOR OTHER RISK FACTORS ARE SHOWN

	Tubal sterilisation	Hysterectomy
Heavy periods		
Yes	0.63 (0.38–1.1)	0.54 (0.35–0.84)
No	0.60 (0.41–0.86)	0.74 (0.50–1.1)
Painful periods		
Yes	0.55 (0.36–0.83)	0.61 (0.42–0.89)
No	0.69 (0.45–1.1)	0.69 (0.44–1.1)

al., 1989; Hankinson *et al.*, 1993; Irwin *et al.*, 1991; Mori *et al.*, 1992; Whittemore *et al.*, 1992). It is unlikely that the protective effect of hysterectomy was explained by inclusion of controls with bilateral oophorectomy since most hysterectomies among controls were validated against medical reports (Green *et al.*, 1997). Risk was low 25 years or more after surgery, discounting previous suggestions (Weiss and Harlow, 1986) that the reduced risk is due to pre-operative screening for malignancy. However, women who had bilateral oophorectomy as well as hysterectomy for pelvic endometriosis would not have been represented among controls, possibly lowering their risk of endometrioid ovarian cancer (Russell, 1994).

Another explanation of the protective effect of tubal surgery is that interruption of trophic utero-ovarian circulation results in fewer ovulations (Hankinson *et al.*, 1993; Whittemore *et al.*, 1992) or hormonal imbalance (Cattanach, 1985). The degree to which the utero-ovarian circulation is compromised by tubal sterilisation varies with the surgical technique, with diathermy of the fallopian tubes expected to interfere with the ovarian circulation more than the application of clips or rings, for example. However, the present data indicate that sterilisation techniques which minimally disturb the ovarian circulation were associated with very low risks of ovarian cancer. Furthermore, neither ovulatory frequency (Rivera *et al.*, 1989) nor hormonal activity (Wu *et al.*, 1992) showed systematic changes after tubal sterilisation. Indeed, gonadal atrophy is associated with enhanced pituitary gonadotrophin production (Oliver, 1990) and may enhance carcinogenesis. Another speculation (Cramer and Xu, 1995) is that the protective effect of tubal occlusion could be explained by a reduction in uterine growth factors reaching the ovaries through the compromised utero-ovarian circulation, but this seems unlikely when removal of the uterus, the source of the growth factors, is not as protective as tubal sterilisation (Cramer and Xu, 1995; Hankinson *et al.*, 1993). Neither is the suggestion that tubal occlusion may block the ascent of carcinogenic infectious agents (Wahlberg, 1994) supported here, nor in studies of oncogenic human papilloma virus (HPV) and ovarian cancer, only one of which (Kaufmann *et al.*, 1987) has detected HPV-6 DNA, in 10 of 12 ovarian cancers.

Our study systematically combined information from women with either tubal sterilisation or hysterectomy, to investigate the effect of tubal closure on risk of ovarian cancer. Potential exposure of the peritoneal epithelium to various agents *via* patent fallopian tubes is of concern because ovarian surface epithelial cells are particularly susceptible to malignant transformation. Not only are these cells prone to molecular genetic errors because of repetitive post-ovulation proliferation but also they behave as generative stem cells, unlike most epithelia, whereby a single mutation can be passed on to exponentially expanding progeny (Godwin *et al.*, 1993). Exposure to vaginal sprays or foams (Silver, 1994) was not associated with risk of ovarian cancer, though use may have been poorly recalled by older women. Talc from condoms (Kasper and Chandler, 1995) or diaphragms may be another peritoneal contaminant, but duration of exposure was not associated with ovarian malignancy in these data. However, use of talc in the perineal region was associated with a significant (30%) increase in risk of ovarian cancer. Again, recall of use of talc among older women may not have been accurate, tending to reduce estimated RRs; moreover, the actual quantity of talc used was unknown. Despite the limitations, these results add support to the body of evidence implicating talc as a factor in the pathogenesis of peritoneal epithelial neoplasia (Cramer *et al.*, 1982; Chen *et al.*, 1992; Longo and Young, 1979; Rosenblatt *et al.*, 1992; Whittemore *et al.*, 1988). Notably, women who had never been regularly exposed to talc in the perineal region and had surgical tubal closure experienced the lowest risk of ovarian cancer, in contrast to those women with patent tubes who were regularly exposed to perineal talc, in agreement with Whittemore *et al.* (1988). Women's usage of talc in the perineal region appears widespread—up to 40% among women in the United States (Whittemore *et al.*, 1988) and Australia alike (Purdie *et al.*, 1995)—so that even an apparently small increase in RR of ovarian cancer associated with perineal talc use would pose a sizable health risk to the population. Talc fibres have been found in normal and malignant ovaries (Henderson *et al.*, 1979). Talc is closely related to and (until recently) variably contaminated by asbestos (Longo and Young, 1979) and may have similar effects on pleural and peritoneal epithelia. Occupational exposures to talc (Kleinfeld *et al.*, 1967), asbestos (Acheson *et al.*, 1982) and rock salt (Tarchi *et al.*, 1994) are significantly associated with ovarian cancer mortality.

Some pelvic contaminants do not appear to have been studied in this context before. Retrograde passage of endometrium is believed to occur in most women with patent fallopian tubes (Halme *et al.*, 1984). We hypothesised that women who report habitual heavy or painful periods experience retrograde menstruation to a greater degree than other women and that this explains the association between heavy or painful periods and ovarian cancer (which is not explained by oral-contraceptive use). We tested this theory by seeking a differential effect of tubal surgery among women according to severity of retrograde menses and found that women who had heavy periods before hysterectomy tended to have a lower risk of ovarian cancer after surgery than women who had average or light periods. This effect was not seen for tubal sterilisation, perhaps because menorrhagia often occurs after tubal sterilisation.

After tubal occlusion, women who reported painful periods also had a lower risk of ovarian cancer than those who had surgery but did not have painful periods. This general tendency may be due merely to chance; alternatively, the differential effect may be real, suggesting that retrograde passage of endometrial fluid is involved. Normal endometrium produces an array of cytokines and growth factors which can stimulate proto-oncogenes and DNA synthesis (Smith, 1991); thus, endometrium in the peritoneal cavity could enhance epithelial tumour development and progression.

Finally, it was hypothesised that if peritoneal irritants do play a causal role in the development of malignancy of the whole peritoneal surface (Woodruff, 1979) and not only that portion overlying the ovaries, then the risk of peritoneal cancers in particular should be substantially reduced after tubal occlusion. This is because the causal mechanism generally proposed for ovarian cancer—namely, the repetitive trauma and repair of the ovarian epithelium (Fathalla, 1971)—would not be implicated for primary peritoneal tumours, leaving exposure to irritants as one of the few likely causes; indeed, a 76% reduction in risk of peritoneal tumours was observed after tubal occlusion. In view of this particular finding and the evidence presented here and elsewhere that pelvic contaminants such as talc are associated with ovarian cancer, we conclude that closure of the fallopian tubes by surgery prevents chronic contact between these agents and ovarian epithelium. It seems likely that peritoneal irritants act as co-carcinogens by increasing the accumulated number of mutational events in ovarian surface epithelial cells.

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REFERENCES

ACHESON, E.D., GARDNER, M.J., PIPPARD, E.C. and GRIME, L.P., Mortality of two groups of women who manufactured gas masks from chrysotile and crocidolite asbestos: a 40-year follow-up. *Brit. J. Indust. Med.*, **39,** 344–348 (1982).

BOOTH, M., BERAL, V. and SMITH, P., Risk factors for ovarian cancer: a case-control study. *Brit. J. Cancer,* **60,** 592–598 (1989).

CATTANACH, J., Oestrogen deficiency after tubal ligation. *Lancet*, **1,** 847–849 (1985).

CHEN, Y., WU, P.C., LANG, J.H., GE, W.J., HARTGE, P. and BRINTON, L.A., Risk factors for epithelial ovarian cancer in Beijing, China. *Int. J. Epidemiol.*, **21,** 23–29 (1992).

CRAMER, D.W., WELCH, W.R., SCULLY, R.E. and WOJCIECHOWSKI, C.A., Ovarian cancer and talc. A case-control study. *Cancer,* **50,** 372–376 (1982).

CRAMER, D.W. and XU, H., Epidemiologic evidence for uterine growth factors in the pathogenesis of ovarian cancer. *Ann. Epidemiol.*, **5,** 310–314 (1995).

FATHALLA, M.F., Incessant ovulation—a factor in ovarian neoplasia? *Lancet*, **2,** 163 (1971).

GODWIN, A.K., TESTA, J.R. and HAMILTON, T.C., The biology of ovarian cancer development. *Cancer,* **71** (Suppl.), 530–536 (1993).

GRAHAM, J. and GRAHAM, R., Ovarian cancer and asbestos. *Environ. Res.*, **1,** 115–128 (1967).

GREEN, A., BAIN, C., SISKIND, V., PURDIE, D., GREEN, L., DICK, M.-L. and THE SURVEY OF WOMEN'S HEALTH STUDY GROUP, Validity of self-reported hysterectomy and tubal sterilization. *Aust. N.Z. J. Publ. Health* (In press) (1997).

HALME, J., HAMMOND, M.G., HULKA, J.F., RAJ, S.G. and TALBERT, L.M., Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet. Gynecol.*, **64,** 151–154 (1984).

HANKINSON, S.E., HUNTER, D.J., COLDITZ, G.A., WILLETT, W.C., STAMPFER, M.J., ROSNER, B., HENNEKENS, C.H. and SPEIZER, F.E., Tubal ligation, hysterectomy, and risk of ovarian cancer. *J. Amer. Med. Ass.*, **270,** 2813–2818 (1993).

HARTGE, P., SCHIFFMAN, M.H., HOOVER, R., MCGOWAN, L., LESHER, L. and NORRIS, H.J., A case-control study of epithelial ovarian cancer. *Amer. J. Obstet. Gynecol.*, **161,** 10–16 (1989).

HENDERSON, W.J., HAMILTON, T.C. and GRIFFITHS, K., Talc in normal and malignant ovarian tissue. *Lancet*, **1,** 499 (1979).

IRWIN, K.L., WEISS, N.S., LEE, N.C. and PETERSON, H.B., Tubal sterilization, hysterectomy, and the subsequent occurrence of epithelial ovarian cancer. *Amer. J. Epidemiol.*, **134,** 362–369 (1991).

KASPER, C.S. and CHANDLER, P.J., Possible morbidity in women from talc on condoms. *J. Amer. med. Ass.*, **273,** 846–847 (1995).

KAUFMAN, R.H., BORNSTEIN, J., GORDON, A.G., ADAM, E., KAPLAN, A.L. and ADLER-STORTHZ, K., Detection of human papilloma virus DNA in advanced epithelial ovarian carcinoma. *Gynecol. Oncol.*, **27,** 340–349 (1987).

KLEINFELD, M., MESSITE, J., KOOYMAN, O. and ZAKI, M.H., Mortality among talc miners and millers in New York State. *Arch. Environ. Health.*, **14,** 663–667 (1967).

LONGO, D.L. and YOUNG, R.C., Cosmetic talc and ovarian cancer. *Lancet*, **2,** 349–351 (1979).

MORI, M., NISHIMURA, H. and FUKUDA, K., Association of tubal sterilization and induced abortion with ovarian cancer. *J. Epidemiol.*, **2,** 111–118 (1992).

OLIVER, R.T.D., Atrophy, hormones, genes and viruses in aetiology of germ cell tumours. *Cancer Surveys*, **9,** 263–268 (1990).

PURDIE, D., GREEN, A., BAIN, C., SISKIND, V., WARD, B., HACKER, N., QUINN, M., WRIGHT, G., RUSSELL, P., SUSIL, B. and SURVEY OF WOMEN'S HEALTH STUDY GROUP, Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. *Int. J. Cancer,* **62,** 678–684 (1995).

RISCH, H.A., MARRETT, L.D. and HOWE, G.R., Parity, contraception, infertility and the risk of epithelial ovarian cancer. *Amer. J. Epidemiol.*, **140,** 585–597 (1994).

RIVERA, R., GAITAN, J.R., RUIZ, R., HURLEY, D.P., ARENAS, M., FLORES, C. and HERNANDEZ, A.B., Menstrual patterns and progesterone circulating levels following different procedures of tubal occlusion. *Contraception,* **40,** 157–169 (1989).

ROSENBLATT, K., SZKLO, M. and ROSENHEIN, N.B., Mineral fiber exposure and the development of ovarian cancer. *Gynecol. Oncol.*, **45,** 20–25 (1992).

RUSSELL, P., Surface epithelial stromal tumors of the ovary. *In:* R.J. Kurman (ed.), *Blaustein's pathology of the genital tract,* Springer-Verlag, New York (1994).

SHU, X.O., BRINTON, L.A., GAO, Y.T. and YAUN, J.M., Population based case-control study of ovarian cancer in Shanghai. *Cancer Res.*, **49,** 3670–3674 (1989).

SILVER, A.-L., Tubal ligation, hysterectomy and risk of ovarian cancer. *J. amer. med. Ass.*, **271,** 1235 (1994).

SMITH, S.K., The endometrium and endometriosis. *In:* E.J. Thomas and J.A. Rock (eds.), *Modern approaches to endometriosis,* Kluwer, Dordrecht (1991).

TARCHI, M., ORSI, D., COMBA, P., DE SANTIS, M., PIRASTU, R., BATTISTA, G. and VALIANI, A., Cohort mortality study of rock salt workers in Italy. *Amer. J. indust. Med.*, **25,** 251–256 (1994).

WAHLBERG, C., Tubal ligation, hysterectomy and risk of ovarian cancer. *J. amer. med. Ass.*, **271,** 1236 (1994).

WEISS, N.S. and HARLOW, B.L., Why does hysterectomy without bilateral oophorectomy influence the subsequence incidence of ovarian cancer? *Amer. J. Epidemiol.*, **124,** 856–858 (1986).

WHITTEMORE, A.S., HARRIS, R., INTYRE, J. and THE COLLABORATIVE OVARIAN CANCER GROUP, Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies II. Invasive epithelial ovarian cancers in white women. *Amer. J. Epidemiol.*, **136,** 1184–1203 (1992).

WHITTEMORE, A.S., WU, M.L., PAFFENBARGER, R.S., SARLES, D.L., KAMPERT, J.B., GROSSER, S., JUNG, D.L., BALLON, S. and HENDRICKSON, M., Personal and environmental characteristics related to epithelial ovarian cancer. II. Exposures to talcum powder, tobacco, alcohol and coffee. *Amer. J. Epidemiol.*, **128,** 1228–1240 (1988).

WOODRUFF, J.D., The pathogenesis of ovarian neoplasia. *Johns Hopkins med. J.*, **144,** 117–120 (1979).

WU, E., BILIAN, X., WEIQIAN, Y., HUI, L. and WU, B., Hormonal profile of the menstrual cycle in Chinese women after tubal sterilization. *Contraception,* **45,** 583–593 (1992).

Exhibit 34

Perineal Talc Exposure and Risk of Ovarian Carcinoma

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BACKGROUND. Clinical and epidemiologic studies have indicated the possible existence of an association between ovarian carcinoma and talcum powder use. Talc particles have been detected in histologic sections of ovarian carcinomas. It has also been demonstrated that inert particles travel from the perineum to the ovaries. Results from epidemiologic investigations have varied, from risks increased by twofold to no significant risk detected.

METHODS. A total of 450 patients with borderline and invasive ovarian carcinoma and 564 population controls in metropolitan Toronto and nearby areas of southern Ontario, Canada, were identified. These subjects were interviewed about their reproductive and menstrual histories as well as their exposure to dusting powders. Continuous unconditional logistic regression methods were used for analysis.

RESULTS. Exposure to talc, via sanitary napkins, direct application to the perineum, or both, was significantly associated with risk of ovarian carcinoma (odds ratio [OR] 1.42, 95% confidence interval [CI] 1.08–1.86). A borderline-significant association was detected between duration of talc exposure and risk (OR 1.09, 95% CI 0.98–1.21, per 10 years of exposure). No significant association was found between frequency of exposure and risk. In comparing invasive and borderline carcinomas, risk remained elevated for both carcinoma types. Only risk for invasive carcinoma was statistically significant.

CONCLUSIONS. This investigation supports previous contentions that exposure to talc may increase risk of ovarian carcinoma. Questionable trends in duration and frequency of exposure suggest that further studies may be needed to clarify the role of talc in the etiology of this disease. ***Cancer* 1997;79:2396–401.**

KEYWORDS: case-control studies, ovarian neoplasms, risk factors, talc.

Ovarian carcinoma is the most commonly fatal gynecologic malignancy.[1] In the United States, approximately 26,000 women develop the disease annually. Overall, the lifetime risk for the development of ovarian carcinoma is 1.4 in 100.[2] Because industrialized nations generally have higher prevalence rates of this disease, environmental exposure has been suggested as an etiologic factor.[1]

Asbestos, a known sclerosing agent, has been shown to cause bronchogenic lung carcinoma and mesothelioma.[3] The incidence of ovarian carcinoma generally increases with greater incidence of asbestosis.[4] Furthermore, female asbestos workers have unusually high numbers of peritoneal neoplasms, and an association between ovarian carcinoma and asbestos exposure has also been observed in animal models.[2,5] Due to its chemical similarity to asbestos, talc has long been suspected as a lung and ovarian carcinogen.[2,6] Like asbestos, talc is a magnesium silicate. Pulverized talc, or talcum powder, is a popular bath and cosmetic product because of its absorbent and

water-repellent properties. Talcum powder is often applied to sanitary napkins and condoms, as well as directly to the perineum, typically after bathing. Early pathology studies have identified talc particles in ovarian tumors.[7,8] An extraction-replication technique developed by Henderson et al.[7] detected talc particles in approximately 75% of ovarian tumors examined. Furthermore, studies of the transport of particles in the human female reproductive tract have shown that nonmotile, inert carbon particles deposited in the vagina can be recovered 30–35 minutes later in the fallopian tubes.[9] Although findings of talc particles in ovarian tumors initially met with skepticism, subsequent evaluations appeared to support the contention of an association between talc exposure and ovarian carcinoma.[10]

In addition to pathologic and clinical studies, several epidemiologic studies have addressed the plausibility of an association between talc and ovarian carcinoma. Although many of these case-control studies revealed elevated risks,[11–18] risk estimates from other studies were not statistically significant[19–20] or were about unity.[21] Thus, the possibility of a risk of ovarian carcinoma that is related to talc exposure remains to be investigated further. The population-based case-control analysis described in this article was conducted to examine the role of talc in ovarian carcinoma, with consideration of the duration, frequency, and method of exposure.

MATERIALS AND METHODS

Study methods have been reported in detail elsewhere[22] and will be summarized here. Our study population consisted of women between the ages of 35 and 79 years residing in the highly populated area surrounding the western end of Lake Ontario, Canada. Cases were women who had histologically confirmed primary, invasive or borderline epithelial ovarian tumors first diagnosed between November 1, 1989, and October 31, 1992. Of the 631 women identified as cases, 450 (71.3%) were interviewed. Fifty-five (8.7%) had died, but proxy interviews were not conducted; 29 (4.6%) had physicians who refused consent; 30 (4.8%) were too ill to participate; 17 (2.7%) were lost to follow-up; and 50 (7.9%) refused to participate.

Population-based controls were identified through the Ontario Ministry of Finance. Information on name, address, age, and gender was obtained from the Enumeration Composite Record listings, which include all homeowners, tenants, and family members, i.e., all persons in the province. From this listing, women living in the study area during the same 3-year period as the cases were randomly selected. Controls were matched to the cases within 3 15-year age groups. Initial contact by letter was followed up with phone calls to determine eligibility. Women with bilateral oophorectomy performed at least 1 year previously were excluded. Overall, 873 eligible controls were identified. Of these women, 564 (64.5%) were interviewed. The remainder either refused to participate (30.2%), were too ill to participate (1.9%), or were lost to follow-up (3.2%).

A questionnaire was developed to detail the medical and reproductive histories of the subjects. This questionnaire was administered during an in-person, in-home interview after informed consent was obtained. The questionnaire focused on menstrual characteristics, pregnancies, and hormone and contraceptive use. Questions about regular talc use and type of talc use, as well as questions from which information about duration and frequency of exposure could be derived, were included. Dusting or powdering behaviors considered included regular application of talc to the perineum after showering or bathing and dusting of talc on sanitary napkins. Parallel information about cornstarch use was also obtained.

Analysis was performed by modeling the data through multiple unconditional logistic regression with the SAS statistical package. In addition to the variables of interest examined here, the models included indicator terms for the age categories of the frequency matching (35–49, 50–64, and 65–79 years), and age as a continuous variable was also included to adjust for residual age effects. Models also contained terms for total years of oral contraceptive use; number of full-term pregnancies; average duration of breastfeeding per pregnancy; and ever having had a tubal ligation, a hysterectomy, or a mother or sister with ovarian or breast carcinoma.

RESULTS

Table 1 shows the descriptive characteristics of the 450 ovarian carcinoma cases and the 564 population controls. Age at interview was used as a matching variable. As observed in many reports,[20,22,23] controls had, on average, a greater number of full-term pregnancies. A higher percentage of controls had had a tubal ligation or a hysterectomy, whereas a higher percentage of cases had a mother or sister with ovarian or breast carcinoma. Years of oral contraceptive use and months of lactation per pregnancy showed trends of decreasing risk with increasing exposure. There were no appreciable differences in the characteristics shown in Table 1 between controls who reported ever having used talc and those not reporting talc use.

Table 2 gives the associations between dusting behaviors and risk of ovarian carcinoma. Overall, 44.0% of cases and 35.6% of controls reported exposure to

TABLE 1
Descriptive Characteristics of Study Population

Characteristics	Cases	Controls	Adjusted[a] OR	Adjusted[a] (95% CI)
Age at interview (yrs)	57.2	57.5	Matched	
Born in Canada or the U.S. (%)	59.1	64.7	0.843	(0.64–1.11)
Race (% black)	1.56	1.95	0.804	(0.30–2.17)
Length of schooling (yrs)	12.3	12.5	0.983	(0.95–1.02)
Number of full-term pregnancies	1.90	2.45	0.820[b]	(0.71–0.92)
Yrs of oral contraceptive use	4.17	5.53	0.915[b]	(0.88–0.95)
Mos of lactation per pregnancy	3.95	4.21	0.946[b]	(0.91–0.99)
Ever had tubal ligation (%)	18.0	24.3	0.659	(0.47–0.93)
Ever had hysterectomy (%)	13.8	24.8	0.485	(0.34–0.69)
Mother/sister with breast or ovarian carcinoma (%)	12.9	7.98	1.917	(1.24–2.97)

OR: odds ratio; CI: confidence interval.

[a] Adjusted for age at interview; yrs of oral contraceptive use; number of full-term pregnancies; average duration of breastfeeding per pregnancy; and ever having had a tubal ligation, hysterectomy, or a mother or sister with ovarian or breast carcinoma.

[b] OR per each, yr or mo, respectively.

talc. Women with any regular talc exposure were at an increased risk (odds ratio [OR] 1.42, 95% confidence interval [CI] 1.08–1.86). The use of cornstarch, or cornstarch sometimes and talc sometimes, did not yield a significant association with risk (cornstarch OR 0.31, 95% CI 0.06–1.66; cornstarch/talc OR 0.68, 95% CI 0.18–2.55). However, application of cornstarch to sanitary napkins or directly to the perineum was not common in this population; less than 2% of the study population reported this behavior. With respect to the type of exposure, substantially more women reported applying talc to their bodies after bathing or showering than using talc on their sanitary napkins. Some 11.3% of cases and 8.7% of controls reported using talc on sanitary napkins. A nonsignificant increase in odds was observed for talc exposure via sanitary napkins (OR 1.26, 95% CI 0.81–1.96). In total, 38.2% of cases and 32.4% of controls reported that they had, at some time, regularly used talc after bathing or showering. The odds ratio seen for use of talc after bathing or showering alone was of borderline statistical significance (OR 1.31, 95% CI 1.00–1.73).

The association between duration and frequency of talc use and ovarian carcinoma was also examined. The mean years of after-bath talc use were 32.9 for cases who had ever used talc after bathing and 35.4 for controls. A borderline-significant trend for years of talc exposure and risk of ovarian carcinoma was found (OR per 10 years of use 1.06, 95% CI 0.99–1.14). When duration was considered categorized by tertiles of control use, only durations of less than 30 years of talc use showed increased risk, relative to no talc exposure. The mean frequency of talc use among those who had ever used it was 14.6 applications per month for cases; for controls, it was 17.2 applications per month. As a continuous variable, monthly frequency did not significantly increase risk of ovarian carcinoma. Categoric analysis of frequency showed that frequencies of less than 10 applications per month may be associated with increased risk; greater frequencies, however, did not show significant increases in risk.

To examine the effects of calendar time of exposure and of hysterectomy or tubal ligation, we assumed that regular after-bath talc use commenced at age 20 years. Table 2 shows that duration of after-bath talc use both before and after 1970 appeared to be associated with risk of ovarian carcinoma. As might be expected, the increased risk seemed to be related mostly to talc use prior to tubal ligation or hysterectomy (Table 2). There were no differences in these results when various starting ages between 15 and 25 years were considered.

The association between talc exposure and invasive ovarian carcinoma, as compared with borderline ovarian carcinoma, was also examined (Table 3). Although the risk remained elevated for both carcinoma types, only the risk for invasive carcinoma was statistically significant. No differences in risk with respect to serous, mucinous, or endometrioid tumors were observed in our data.

Substantial alteration in risk of ovarian carcinoma was not observed for general sanitary napkin use com-

TABLE 2
Risk of Ovarian Carcinoma with Use of Talcum Powder or Cornstarch

	No. (%)				Adjusted[b]	
	Cases	Controls	Case mean[a]	Control mean[a]	OR	(95% CI)
Any talc exposure	198 (44.0)	201 (35.6)			1.420	(1.08–1.86)
Any cornstarch	2 (0.44)	5 (0.85)			0.305	(0.06–1.66)
Cornstarch/talc	4 (0.89)	7 (1.24)			0.681	(0.18–2.55)
Type of talc exposure						
Sanitary napkin	51 (11.3)	49 (8.69)			1.262	(0.81–1.96)
After bathing	172 (38.2)	183 (32.4)			1.312	(1.00–1.73)
After-bath talc use/mo			14.6	17.2	0.890[c]	(0.74–1.07)
<10	76 (16.9)	59 (10.5)			1.836	(1.24–2.73)
10–25	54 (12.0)	64 (11.3)			1.128	(0.74–1.72)
>25	41 (9.11)	60 (10.6)			0.951	(0.61–1.49)
Yrs of after-bath talc use			32.9	35.4	1.091[c]	(0.98–1.21)
<30	60 (13.3)	52 (9.22)			1.697	(1.09–2.64)
30–40	71 (15.8)	67 (11.9)			1.435	(0.96–2.15)
>40	41 (9.11)	64 (11.3)			0.865	(0.54–1.38)
Yrs of after-bath talc use						
Before 1970			26.4	24.9	1.090[c]	(0.98–1.22)
After 1970			6.5	10.4	1.095[c]	(0.89–1.35)
Yrs of after-bath talc use						
Before tubal ligation/hysterectomy			28.4	26.9	1.105[c]	(0.99–1.24)
After tubal ligation/hysterectomy			4.5	8.5	1.031[c]	(0.82–1.29)

OR: odds ratio; CI: confidence interval.

[a] Mean among those who had ever used talc.

[b] Adjusted as in Table 1.

[c] OR for the continuous variable, shown per 10 applications per mo or 10 yrs of use, as appropriate.

TABLE 3
Risk of Ovarian Carcinoma for Women Who Ever Used Talcum Powder Regularly, by Case Histology

Histologic type	Total no. of cases	No. (%) who used talcum powder	Adjusted[a] OR	(95% CI)
Invasive	367	166 (45.2)	1.513	(1.13–2.02)
Borderline	83	32 (38.6)	1.237	(0.76–2.02)
Serous	254	109 (42.9)	1.336	(0.96–1.85)
Mucinous	80	35 (43.8)	1.585	(0.97–2.58)
Endometrioid	74	36 (48.6)	1.671	(1.00–2.79)

OR: odds ratio; CI: confidence interval.

[a] Adjusted as in Table 1.

pared with tampon use. Because few women used sanitary napkins or tampons exclusively, the risk was examined as a percentage of the length of time that sanitary napkins were used and a percentage of the length of time that tampons were used. Significant trends in risk were not detected for a 10% difference in napkin use (OR 1.06, 95% CI 0.99–1.13) or for a 10% increase in tampon use (OR 0.99, 95% CI 0.93–1.05).

DISCUSSION

Results from experimental and epidemiologic studies conducted thus far indicate a possible association between talc exposure and ovarian carcinoma. Histologic evidence first indicated that contaminants such talc may become embedded in ovarian tumors.[8,24] Experimental studies have shown that external talc exposure may eventually reach the ovaries. Henderson et al.[10] demonstrated that talc was present in ovaries after deposition of a talc suspension in the vagina and cervical os in rats. Similarly, Egli and Newton[9] revealed in human studies that inert carbon particles deposited in the vagina can later be recovered in the fallopian tubes. Although these studies demonstrated a possible route of exposure to talc, they were nevertheless unable to address the effects of long term talc use.

Surprisingly, few subsequent pathologic and clinical studies have been conducted. Epidemiologic studies addressing the possible association between talc and ovarian carcinoma have generally reported increased risk estimates. Cramer et al.[11] found a relative risk of 1.92 (95% CI 1.3–2.9). Rosenblatt et al.[12] reported a relative risk of 2.4 (95% CI 1.1–5.3) for any genital talc exposure. Likewise, Purdie et al. found a

significant positive association between talc and ovarian carcinoma,[14] and other recent studies also support the hypothesis of elevated risk of ovarian carcinoma with talc exposure, reporting risk increases of approximately two-fold.[16–18] A few studies have found only marginally significant or nonsignificant elevations in risk.[19–21] However, in investigations such as that reported by Tzonou et al., the number of women who reported talc usage was low.[21] More detailed discussion of many of those studies may be found in reviews elsewhere.[25,26]

This study sought to elucidate further the relationship between talc and ovarian carcinoma. Talc exposure through direct perineal application and via sanitary napkins, the frequency and duration of exposure, and the effect of talc within specific histologic subtypes were examined. Dusting with talcum powder was common behavior for more than one-third of cases and controls. The primary mode of talc exposure appeared to be direct application to the perineum. Although talc exposure via contraceptives such as condoms and diaphragms has been previously investigated,[27] this type of behavior was rare in the current study population and therefore omitted from analyses. Overall, greater risk was associated with any regular talc exposure (OR 1.42, 95% CI 1.08–1.86). Any talc exposure included talc accumulated from sanitary napkins, from powdering after bathing, or from both behaviors. Talc exposures via sanitary napkin alone or after bathing conveyed similar magnitudes of increased risk.

Commercial talc substitutes often replace talc with cornstarch. Furthermore, women may choose to powder or dust with cornstarch instead of talc. When cornstarch was assessed in relation to risk of ovarian carcinoma, no associations were found. This suggests that the association between talc use and risk of ovarian carcinoma may not be due simply to a difference in focus on hygiene between cases and controls. Use of cornstarch, however, was rare in our population, as less than 1% of the cases and controls reported use of cornstarch alone, and very few cases and controls reported use of cornstarch sometimes and talc sometimes.

A questionable dose-response relationship was observed between duration or frequency of exposure and risk. Duration as a continuous variable indicated that risk may increase with increasing years of talc exposure. These results are similar to findings by Cramer et al.,[11] Harlow et al.,[13] Harlow and Weiss,[28] Cook et al.,[18] and Whittemore et al.,[19] in which trends of duration and frequency were not significant. Booth et al.[23] reported a marginally significant trend with frequency. It is noteworthy that exposures of less than 30 years, at frequencies of less than 10 applications per month, and prior to tubal ligation or hysterectomy showed the most significant elevations in risk in the current study.

When the outcome, ovarian carcinoma, was further segregated into invasive and borderline carcinomas, talc exposure was associated with both but was only significant for invasive carcinomas. This result contrasts with the observations of Harlow et al.,[13] who found the strongest talc–ovarian carcinoma associations among women with endometrioid and borderline tumors. Cook et al.[18] reported no increase in risk of mucinous tumors; this was similar to our observation that mucinous tumors may not be associated with other ovarian carcinoma risk factors.[29] An earlier study, however, found no variation in risk by histologic subtype,[11] and the current study also found no differences in talc use associated with serous, mucinous, or endometrioid tumors.

Several lines of evidence support the argument for an association between talc usage and ovarian carcinoma. Talc and asbestos are chemically related; and although asbestos contamination in talc products has been closely regulated, talc and asbestos are frequently found together in mining strata. Asbestos is a known cause of pleural and peritoneal mesotheliomas, which are histologically similar to ovarian carcinomas.[19] Two possible mechanisms have been suggested for the role of talc in the etiology of ovarian carcinoma. With ovulation, entrapment of the ovarian epithelium within the stroma occurs. During this time, talc, if present, may become incorporated into these inclusion cysts, providing a favorable environment for carcinogenesis.[11] Alternatively, talc may serve to stimulate the entrapment of the surface epithelium and may act in a manner similar to ''incessant ovulation,'' which has been proposed as an etiologic factor in ovarian carcinoma.[11,30]

Differences in talc concentration among various baby powders, body powders, and deodorizing powders were not investigated in this study. Furthermore, reporting error in reported talc use and failure to interview all eligible case and control subjects may also have led to biases. As with any case-control study, the possibility of selection bias and information bias exists, although the consistency of this study with others that have addressed reproductive factors and ovarian carcinoma is reassuring.[23] Further discussion of the strengths and weaknesses of the current study may be found in a previous report.[23]

The results of this study appear to support the contention that talc exposure increases risk of ovarian carcinoma. Dusting with talcum powder is not an unusual practice for women, and, given the heterogeneity

of the etiology and course of ovarian carcinoma, any possible harmful practices, particularly those with little benefit, should be deliberated. It should be emphasized, however, that further studies are needed to clarify the role of talc in the etiology of ovarian carcinoma.

REFERENCES

1. Longo DL, Young RC. Cosmetic talc and ovarian cancer. *Lancet* 1979;2:1011–2.
2. Herbst AL. The epidemiology of ovarian carcinoma and the current status of tumor markers to detect disease. *Am J Obstet Gynecol* 1994;170:1099–107.
3. Keal EE. Asbestosis and abdominal neoplasms. *Lancet* 1960;2:1211–6.
4. Graham J, Graham R. Ovarian cancer and asbestos. *Environ Res* 1967;1:115–28.
5. Acheson ED, Gardner MJ, Pippard EC, Grime LP. Mortality of two groups of women who manufactured gas masks from chrysotile and crocidolite asbestos: a 40-year follow-up. *Br J Ind Med* 1982;39:344–8.
6. Kleinfeld M, Messite J, Zaki MH. Mortality experience among talc workers: a follow-up study. *J Occup Med* 1974;16:345–9.
7. Henderson WJ, Joslin CAF, Turnbull AC, Griffiths K. Talc and carcinoma of the ovary and cervix. *J Obstet Gynecol Br Commonw* 1971;78:266–72.
8. Griffiths K, Henderson WJ, Chandler JA, Joslin CAF. Ovarian cancer: some new analytical approaches. *Postgrad Med* 1973;49:69–72.
9. Egli GE, Newton M. The transport of carbon particles in the human female reproductive tract. *Fertil Steril* 1961;12:151–5.
10. Henderson WJ, Hamilton RC, Griffiths K. Talc in normal and malignant ovarian tissue. *Lancet* 1979;1:499.
11. Cramer DW, Welch WR, Scully RE, Wojciechoski CA. Ovarian cancer and talc: a case-control study. *Cancer* 1982;50:372–6.
12. Rosenblatt KA, Szklo M, Rosenshein NB. Mineral fiber exposure and the development of ovarian cancer. *Gynecol Oncol* 1992;45:20–5.
13. Harlow BL, Cramer DW, Bell DA, Welch WR. Perineal exposure to talc and ovarian cancer risk. *Obstet Gynecol* 1992;80:19–26.
14. Purdie D, Green A, Bain C, Siskind V, Ward B, Hacker N, et al. Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. *Int J Cancer* 1995;6:678–84.
15. Hartge P, Stewart P. Occupation and ovarian cancer: a case-control study in the Washington DC, Metropolitan Area, 1978–1981. *J Occup Med* 1994;36:924–7.
16. Cook LS, Kamb ML, Weiss NS. Perineal powder exposure and the risk of ovarian cancer. *Am J Epidemiol* 1996;143:S83.
17. Shushan A, Paltiel O, Iscovich J, Elchalal U, Peretz T, Schenker JG. Human menopausal gonadotropin and the risk of epithelial ovarian cancer. *Fertil Steril* 1996;65:13–8.
18. Cook LS, Kamb ML, Weiss NS. Perineal powder exposure and the risk of ovarian cancer. *Am J Epidemiol* 1997;145:459–65.
19. Whittemore AS, Wu ML, Paffenbarger RS Jr., Sarles DL, Kampert JB, Grosser S, et al. Personal and environmental characteristics related to epithelial ovarian cancer. *Am J Epidemiol* 1988;128:1228–40.
20. Chen Y, Wu P-C, Lang J-H, Ge W-J, Hartge P, Brinton LA. Risk factors for epithelial ovarian cancer in Beijing, China. *Int J Epidemiol* 1992;21:23–9.
21. Tzonou A, Polychronopoulou A, Hsieh CC, Rebelakos A, Karakatsani A, Trichopoulos D. Hair dyes, analgesics, tranquilizers and perineal talc application as risk factors for ovarian cancer. *Int J Cancer* 1993;55:408–10.
22. Risch HA, Marrett LD, Howe GR. Parity, contraception, infertility, and the risk of epithelial ovarian cancer. *Am J Epidemiol* 1994;140:585–97.
23. Booth M, Beral V, Smith P. Risk factors for ovarian cancer: a case-control study. *Br J Cancer* 1989;60:592–8.
24. Henderson WJ, Hamilton TC, Baylis MS, Pierrepoint CG, Griffiths K. The demonstration of the migration of talc from the vagina and posterior uterus to the ovary in the rat. *Environ Res* 1986;40:247–50.
25. Gross AJ, Berg PH. A meta-analytical approach examining the potential relationship between talc exposure and ovarian cancer. *J Expo Anal Environ Epidemiol* 1995;5:181–95.
26. Harlow BL, Hartge PA. A review of perineal talc exposure and risk of ovarian cancer. *Regul Toxicol Pharmacol* 1995;21:254–60.
27. Kasper CS, Chandler PJ Jr. Possible morbidity in women from talc on condoms. *JAMA* 1995;273:846–7.
28. Harlow BL, Weiss NS. A case-control study of borderline ovarian tumors: the influence of perineal exposure to talc. *Am J Epidemiol* 1989;130:390–4.
29. Risch HA, Marrett LD, Jain M, Howe GR. Differences in risk factors for epithelial ovarian cancer by histologic type: results of a case-control study. *Am J Epidemiol* 1996;144:363–72.
30. Fathalla MF. Factors in causation and incidence of ovarian cancer. *Obstet Gynecol Surv* 1972;27:751–68.

Exhibit 35

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Perineal Powder Exposure and the Risk of Ovarian Cancer

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This case-control study evaluated the risk of epithelial ovarian cancer associated with genital exposure to various forms of powder application. Cases included all women aged 20–79 years in three counties of western Washington who were diagnosed with borderline or invasive ovarian cancer from 1986 through 1988; 64.3% of eligible cases were interviewed. A sample of similarly aged women who lived in these counties, identified by random digit dialing, served as controls. The overall response among control women was 68.0%. Information on powder application and other potential risk factors was ascertained during the in-person interview. Overall, ovarian cancer cases (n = 313) were more likely than controls (n = 422) to ever have used powder (age-adjusted relative risk (RR) = 1.5, 95% confidence interval (CI) 1.1–2.0). After adjustment for age and other methods of genital powder application (none vs. any), an elevated relative risk of ovarian cancer was noted only for women with a history of perineal dusting (RR = 1.6, 95% CI 1.1–2.3) or use of genital deodorant spray (RR = 1.9, 95% CI 1.1–3.1) These results offer support for the hypothesis, raised by prior epidemiologic studies, that powder exposure from perineal dusting contributes to the development of ovarian cancer, and they suggest that use of genital deodorant sprays may do so as well. Limitations of the present study include the fairly low proportion of eligible women who participated and the potential differential recall of powder usage. *Am J Epidemiol* 1997;145:459–65.

ovarian neoplasms; powders; talc

Studies documenting the migration of carbon particles and radioactive particulate agents from the vagina to the ovaries (1, 2), as well as those that have identified talc-like particles more frequently in ovarian tumors than in normal human ovarian tissue (3), have raised concern that genital powder exposure may increase a woman's risk of developing ovarian cancer. While the results of several epidemiologic studies have suggested elevated risks for ovarian cancer among women with genital powder exposures (4–11), results have been inconsistent for particular methods of powder application (12). In this population-based case-control study, information on the method, duration, and frequency of powder application was collected to evaluate the impact of genital powder exposures on the risk of epithelial ovarian cancer.

MATERIALS AND METHODS

Women with invasive or borderline epithelial ovarian cancer were identified from records of the population-based Cancer Surveillance System of western Washington. Eligible case subjects included white women diagnosed between January 1, 1986, and December 31, 1988, who resided in three counties of western Washington (King, Pierce, and Snohomish counties) and were 20–79 years of age at diagnosis. After obtaining permission from their personal physicians to contact the women and obtaining written, informed consent, we successfully interviewed 329 (64.3 percent) of the 512 eligible case subjects. The remaining 183 women were not interviewed because of death prior to study contact (n = 104, 20.3 percent), physician or subject refusal (n = 73, 14.3 percent), and lack of success in locating the women (n = 6, 1.2 percent). Seven women whose self-reported race/ethnicity was other than white and nine women with unknown genital powder use were also excluded. Thus, a total of 313 white women diagnosed with borderline (n = 79) or invasive (n = 234) epithelial ovarian tumors were available for analysis.

Women identified as control subjects for this study were part of a larger control pool selected by random digit dialing (13) for several studies of cancer in women. Of the total 10,109 calls made by random digit dialing, 5,853 (57.9 percent) were to nonresidential phone numbers, 3,830 (37.9 percent) were to residential phone numbers, and 426 (4.2 percent) were to

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Abbreviations: CI, confidence interval; RR, relative risk

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numbers of unknown residential status; 3,604 (94.1 percent) of the 3,830 calls to residential households were screened for eligible women who were age matched, in 5-year age groups, to the combined female cancer case group. Of the 721 women identified who were eligible, 521 (72.3 percent) were successfully interviewed after written, informed consent was obtained. The overall response (random digit dialing screening response multiplied by the interview response) was 68.0 percent. Women who reported race/ethnicity other than white ($n = 28$), age greater than 79 ($n = 5$), a history of bilateral oophorectomy ($n = 58$), uncertainty concerning a history of bilateral oophorectomy ($n = 4$), and unknown genital powder use ($n = 4$) were excluded, resulting in a total of 422 white control women for analysis.

Information regarding genital powder exposures was collected by structured, in-person interviews. Women were queried about storing diaphragms in powder, dusting perineal areas with powder after bathing, powdering sanitary napkins, and using genital deodorant sprays (which may contain aerosolized powder). Those who answered affirmatively were questioned further about the duration and frequency of powder application and about the types of powder applied. Powders were grouped into five categories: cornstarch, talcum powder, baby powder, deodorant powder, and scented body/bath powder. Information on demographic characteristics, reproductive history, medical and screening histories, smoking history, anthropometry, and birth control methods was also provided by the women. A calendar was used to record major life events and enhance recall of past exposures. Relevant study information was recorded only for exposures that occurred prior to the diagnosis date of cancer among the cases or the analogous reference date among controls.

Logistic regression (EGRET version 26.6; Statistics and Epidemiology Research Corporation, Seattle, Washington) was used to determine odds ratios as estimates of the relative risk for ovarian cancer associated with genital powder application and 95 percent confidence intervals (14). For all the relative risk estimates reported in the present analysis, women who reported any method, type, or frequency of genital powder application were compared with women who stated that they had never applied genital powder in any manner (154 ovarian cancer cases and 256 controls). Trends were evaluated using the likelihood ratio statistic (14).

First, the relative risk for ovarian cancer among women who reported exclusive use of one of the four methods of powder application was assessed (table 2). Then, because many women used more than one method of powder application, the risk for ovarian cancer among women who reported any use of the four methods of powder application was assessed while adjusting for the other methods of powder application (table 3). Similarly, ovarian cancer risk by exclusive and nonexclusive use of the type(s) of powder used for perineal dusting, diaphragm storage, or on sanitary napkins was assessed (table 4). To assess the impact of genital powder exposure on the risk of specific histologic categories of ovarian tumors (table 5), we grouped borderline and invasive ovarian tumors according to the following *International Classification of Diseases for Oncology* histologic codes (15): serous tumors (codes 8441, 8442, 8460, 8461, and 8462); mucinous tumors (codes 8470, 8472, 8473, 8480, and 8481); endometrioid tumors (codes 8380, 8381, and 8560); and other tumors that included clear cell (code 8310), undifferentiated (code 8020), and unclassified/other (codes 8010, 8050, 8140, 8240, 8260, 8440, 8450, and 9000). All relative risk estimates were adjusted for age. Further adjustment for education, income, marital status, body mass index (weight (kg)/height $(m)^2$), oral contraceptive use, or parity did not alter the estimated relative risks. Information on lactation was not available. Separate analyses for women diagnosed with invasive ovarian cancer and for those diagnosed with borderline ovarian cancer produced results very similar to those presented in tables 2–5.

RESULTS

Selected characteristics of ovarian cancer cases and controls are presented in table 1. Less education, a lower household income, and a higher body mass index were more common among women with ovarian cancer than among control women, but oral contraceptive use and having had a full-term birth were less common.

Genital powder application was more common among cases (50.8 percent) than controls (39.3 percent) (table 2). There was an overall 50 percent elevation in the risk for ovarian cancer associated with the use of one or more of the four possible methods of genital powder application (95 percent CI 1.1–2.0). Among women who exclusively used a single method of powder application, ovarian cancer risk was most strongly elevated among those who dusted perineal areas with powder after bathing (RR = 1.8, 95 percent CI 1.2–2.9).

We further examined ovarian cancer risk among women who reported application of genital powders using each of the four methods, although not necessarily exclusive use of any method (table 3). Perineal dusting was associated with an increased risk of ovar-

TABLE 1. Characteristics of epithelial ovarian cancer cases and controls: King, Pierce, and Snohomish counties, Washington State, 1986–1988

Characteristic	Cases (n = 313)		Controls (n = 422)	
	No	%	No	%
Age (years)				
20–34	34	10 9	84	19.9
35–44	50	16 0	136	32 2
45–54	60	19 2	65	15 4
55–64	88	28 1	63	14 9
65–79	81	25 9	74	17.5
Education (years)				
≤8	15	4.8	14	3.3
9–12	124	39 6	144	34.1
13–16	146	46 6	219	51 9
>16	27	8 6	45	10 7
Unknown	1	0 3	0	
Annual household income ($)				
<15,000	90	28 8	83	19.7
15,000–30,000	91	29 1	153	36 3
>30,000–45,000	60	19 2	81	19 2
>45,000	63	20 1	96	22.7
Unknown/refused	9	2 9	9	2 1
Marital status				
Single	32	10.2	33	7.8
Married	186	59.4	292	69 2
Separated/divorced/widowed	95	30 4	97	23 0
Body mass index (kg/m^2)				
<21	56	17 9	97	23.0
21–22	89	28 4	145	34 4
23–24	73	23 3	75	17.8
≥25	95	30 4	105	24 9
Oral contraceptive use				
Never or ≤12 months	224	71 6	221	52.4
>12 months but <5 years	50	16 0	93	22 0
≥5 years	39	12 5	108	25.6
Total pregnancies				
0	57	18.2	56	13.3
1	42	13 4	56	13.3
≥2	214	68 4	309	73 2
Unknown	0		1	0 2
Total full-term births				
0	79	25 2	83	19.7
1	46	14.7	69	16.4
≥2	188	60.1	269	63.7
Unknown	0		1	0.2

ian cancer (RR = 1.6, 95 percent CI 1.1–2.3), although there was no clear pattern of increasing risk with increasing duration of use. When the small contribution of perineal dusting after a hysterectomy or tubal ligation was excluded from the analysis, our relative risk estimates were nearly unchanged (data not shown). In 1976, the cosmetic industry proposed voluntary guidelines to limit contamination of consumer powders (16), and we attempted to evaluate ovarian cancer risk associated with any perineal dusting in 1976 or before and with exclusive perineal dusting in 1977 or thereafter. Women with any perineal dusting in 1976 or before had an elevated risk (RR = 1.8, 95 percent CI 1.1–2.9), but we were unable to evaluate exclusive perineal dusting in 1977 and thereafter since only four cases and 10 controls had this exposure. The use of genital deodorant sprays was also associated with an elevated ovarian cancer risk (RR = 1.9, 95 percent CI 1.1–3.1), with the strongest elevation in risk among the small number of women (n = 15) who used these sprays for more than 1 year (RR = 2.7, 95 percent CI 1.1–6.6). Storing a diaphragm in powder or powdering sanitary napkins was not related to the risk of developing an ovarian tumor (RR = 1.0, 95 percent CI 0.6–1.6, and RR = 0.9, 95 percent CI 0.5–1.5, respectively).

TABLE 2. Relative risk of epithelial ovarian cancer associated with any genital powder use and by exclusive use of various methods of powder application: King, Pierce, and Snohomish counties, Washington State, 1986–1988

Powder application	Ovarian cancer cases (n = 313)		Controls (n = 422)		RR*	95% CI*
	No	%	No	%		
Lifetime genital powder application						
None	154	49.2	256	60.7	1.0	Referent
Any	159	50.8	166	39.3	1.5	1 1–2.0
Exclusive use of						
Perineal dusting only	55	17.6	48	11.4	1.8	1.2–2.9
Diaphragm storage in powder only	22	7.0	35	8.3	0 8	0.4–1.4
Powder on sanitary napkins only	12	3.8	10	2 4	1.5	0.6–3.6
Genital deodorant spray only	18	5.8	28	6.6	1 5	0 8–3.0

* RR, relative risk, adjusted for age, CI, confidence interval

No specific type of powder used for perineal dusting, diaphragm storage, or on sanitary napkins was strongly related to ovarian cancer risk, although there was a suggestion of an elevated risk associated with any use of talcum powder and bath/body powders (RR = 1.6, 95 percent CI 0.9–2.8, and RR = 1.5, 95 percent CI 0.9–2.4, respectively) (table 4). When specific histologic categories of ovarian tumors were examined, any genital powder application was associated with an elevated risk for serous tumors (RR = 1.7, 95 percent CI 1.1–2.5) and the nonspecific category of other tumors (RR = 1.8, 95 percent CI 1.1–2.8), whereas no elevation in risk was noted for the small number of women with mucinous tumors (RR = 0.7, 95 percent CI 0.4–1.4) or endometrioid tumors (RR = 1.2, 95 percent CI 0.6–2.3) (table 5).

DISCUSSION

There are several issues that should be considered in the interpretation of our results. A sizable number of women eligible for our study did not participate, particularly among those with ovarian cancer. Many women with cancer died before they could be approached about participation in this study, and others were too ill to participate. If substantial differences in powder use existed between participating and nonparticipating women, our study results may over- or underestimate the true risks for ovarian cancer. It is also possible that the completeness of the reporting of powder use differed between cases and controls, biasing our relative risk estimates to some degree.

Additionally, it is not clear how well ascertainment of perineal powder application correctly estimates actual exposure to particles in powder that may influence ovarian cancer risk. Different consumer brands of powder that women used, or even different lots of the same brand, may have varied substantially in the content of talc, asbestiform minerals, or structurally similar compounds. Powder content has also varied over time, presumably with fewer asbestiform minerals present in more recently manufactured products (17–19).

Our results suggest that a history of perineal dusting or use of genital deodorant sprays has a modest influence on the development of epithelial ovarian tumors, whereas storing a diaphragm in powder or powdering sanitary napkins does not. Direct comparisons of our results with those of the other nine published studies (and among these studies) are somewhat limited because of differences in the definitions, groupings, and analysis of genital powder use. Nonetheless, there is some consistency in results among studies. Seven studies including the present one (4, 6, 8–11) reported elevated relative risks for ovarian cancer, ranging from 1.3 to 3.9, among women with powder exposure by "dusting of the perineum." Of the three remaining studies that evaluated the more general exposure of "talc use in genital/perineal area" (which may or may not include perineal, sanitary napkin, diaphragm, or undergarment applications), two observed a modest elevation in ovarian cancer risk (5, 7), whereas one did not (20).

Most studies including the present one have found little, if any, excess risk for ovarian cancer among women who stored their diaphragms in powder (4–8, 10); only one study has reported a suggestion of an elevation in risk (11). In the present study, control women more frequently reported washing their diaphragms prior to use than did ovarian cancer cases, but ovarian cancer risk was not substantially elevated for the small number of women who did not wash their diaphragms prior to use. The relation between powdering sanitary napkins and ovarian cancer risk is less clear; three studies including the present study found

TABLE 3. Relative risk of epithelial ovarian cancer associated with genital powder use by methods of powder application: King, Pierce, and Snohomish counties, Washington State, 1986–1988*

Lifetime genital powder application	Ovarian cancer cases (n = 313)		Controls (n = 422)		RR†	95% CI†
	No	%	No	%		
None	154	49 2	256	60.7	1.0	Referent
Any perineal dusting	95	30 4	87	20 6	1.6	1.1–2 3
Cumulative lifetime days						
≤2,000	20	6 4	22	5 2	1.8	0 9–3.5
2,001–5,000	24	7 7	26	6.2	1.6	0.9–2 9
5,001–10,000	21	6.7	22	5 2	1.2	0 6–2 4
>10,000	28	8.9	17	4 0	1.8	0.9–3.4
Unknown	2	0.6	0			
Diaphragm storage in powder	46	14 7	51	12.1	1 0	0.6–1 6
Cumulative lifetime months						
≤60	24	7.7	26	6 2	1 1	0 6–1.9
>60	15	4 8	20	4.7	0 8	0.4–1 7
Unknown	7	2 2	5	1.2		
Usually washed before use						
No	19	6 1	14	3 3	1.4	0 7–3 0
Yes	20	6.4	31	7 3	0 7	0 4–1 4
Unknown	7	2 2	6	1.4		
Any powder on sanitary napkins	38	12.1	40	9.5	0 9	0.5–1 5
Cumulative lifetime months						
≤120	25	8 0	21	5.0	1 3	0 7–2.4
>120	12	3.8	19	4.5	0 5	0.2–1 1
Unknown	1	0 3	0			
Lifetime applications						
≤1,000	23	7.3	19	4 5	1.3	0.7–2 5
>1,000	14	4 5	21	5.0	0 6	0.3–1 2
Unknown	1	0.3	0			
Any genital deodorant spray	40	12.8	40	9.5	1.9	1.1–3 1
Cumulative lifetime months						
≤12	24	7.7	31	7 4	1.5	0 9–2 8
>12	15	4 8	9	2 1	2.7‡	1.1–6 6
Unknown	1	0 3	0			
Lifetime applications						
≤500	29	9.3	34	8 1	1.7	1 0–2.9
>500	10	3.2	6	1.4	2 6‡	0.9–7 6
Unknown	1	0.3	0			

* Numbers do not add up to total cases and controls because women may have used a variety of methods for powder application.

† RR, relative risk, adjusted for age and for the other methods of genital powder application (none, any), CI, confidence interval.

‡ p value for trend < 0.05.

no association (6, 10), whereas three other studies reported moderate elevations in risk (4, 8, 11).

Only two other studies have evaluated particular types of powder; one reported an excess risk of borderline ovarian tumors among women who used deodorant powders (8), and another study reported an excess risk of ovarian cancer among women who used baby powders (10). A strong relation between the types of powder used and ovarian cancer risk was not found in the present study, although there was a suggestion of an elevated risk with any use of talcum powder and bath/body powders among women using these powders for perineal dusting, diaphragm storage, or on sanitary napkins.

The present study is the first to evaluate the association between genital deodorant spray use and ovarian cancer risk; these preliminary results require confirmation in other studies. It is difficult to postulate that an increased risk for ovarian cancer may specifically be due to powder and associated constituents when some of the deodorant sprays do not contain aerosolized powder. It is possible that it is not powder per se

TABLE 4. Relative risk of epithelial ovarian cancer associated with type of powder used with perineal dusting, diaphragm storage, or sanitary napkins: King, Pierce, and Snohomish counties, Washington State, 1986–1988

Type of powder	Ovarian cancer cases (n = 313)		Controls (n = 422)		RR*	95% CI*
	No	%	No	%		
Lifetime use (none)	154	49 2	256	60.7	1.0	Referent
Exclusive use of						
Talcum powder only	16	5 1	16	3.8	1.2†	0.6–2.5
Baby powder only	31	9 9	36	8.5	1.4†	0.8–2.4
Cornstarch only	5	1 6	11	2.6	0.9†	0.3–2.9
Deodorizing powder only	9	2 9	10	2.4	1 0†	0.4–2.6
Bath/body powder only	27	8 6	25	5.9	1.6†	0.9–3 0
Unspecified type only	11	3 5	4	0.9		
Use of‡						
Any talcum powder	33	10.5	23	5 5	1.6§	0 9–2.8
Any baby powder	52	16.6	61	14.5	1.1§	0.7–1 8
Any cornstarch	8	2.6	16	3 8	0 8§	0.3–2 0
Any deodorizing powder	24	7.7	24	5 7	1.1§	0.6–2.0
Any bath/body powder	52	16 6	43	10 2	1.5§	0.9–2.4
Any unspecified type	24	7 7	11	2.6		

* RR, relative risk; CI, confidence interval.
† Adjusted for age
‡ Numbers do not add up to total cases and controls with any powder use because women may have used a variety of powders
§ Adjusted for age and the other types of powders used (yes, no).

TABLE 5. Relative risk of epithelial ovarian cancer associated with any genital powder use by tumor histology: King, Pierce, and Snohomish counties, Washington State, 1986–1988

Histologic type	Any powder application		No powder application		RR*	95% CI*
	No	%	No	%		
Controls	166	39.3	256	60.7	1 0	Referent
Serous tumors (n = 131)	71	54.2	60	45 8	1.7	1.1–2.5
Mucinous tumors (n = 43)	14	32.6	29	67.4	0.7	0.4–1.4
Endometrioid tumors (n = 36)	17	47.2	19	52.8	1.2	0.6–2 3
Other tumors† (n = 103)	57	55.3	46	44.7	1.8	1.1–2 8

* RR, relative risk, adjusted for age; CI, confidence interval
† Other tumors include 17 clear cell, three undifferentiated, and 83 unclassified (adenocarcinoma or unspecified carcinoma) tumors

but other unidentified chemical substances present in deodorant sprays that may influence the development of ovarian cancer.

A partner's use of condoms that were packed in talc could also have contributed to a woman's genital powder exposure (21). There was insufficient information in the present study to address the influence of condom use on the risk for ovarian cancer. Seven (2.2 percent) ovarian cancer cases and 19 (4.5 percent) control women reported a history of exposure to condoms packed in talc, whereas 20 (6.4 percent) cases and 34 (8.1 percent) controls did not know if their partners had used condoms packed in talc. Furthermore, few women knew or remembered the brand of condoms their partners had used.

The specific constituent(s) of powders that may influence the development of ovarian cancer is unknown, although attention has been focused on fibrous talc particles and asbestos (17–19, 22). Talc, a hydrous magnesium silicate, is a constituent of almost all body and baby powders except for those that are specifically labeled as talc free or pure cornstarch. The nonfibrous, sheet-like layers of talc in these powders slide across each other, allowing a smooth application on the skin. Talc-based powders may also contain fibrous particles, most of which are talc fibers, but some can be asbestiform fibers (17, 18). While pure talc is relatively nontoxic, adverse health effects can include induction of talc granulomas when introduced in open wounds and, in the occupational setting, pneumoconi-

osis (talcosis) in individuals with long-term exposure to talc dust (19). Occupational exposure to talc does not appear to increase the risk for pulmonary malignancies (19). Most animal studies confirm this, with lung tumors developing only in rats exposed to doses of talc dust high enough to cause chronic obstructive and restrictive lung toxicity (19). Excess ovarian tumors have not been reported in rats and mice with long-term exposure to aerosol talc (23). In contrast, occupational exposure to asbestos fibers has been shown to cause lung tumors (24) and has been associated with the development of ovarian tumors (25). Thus, while there is little biologic or experimental evidence to support a role for talc per se in the development of ovarian malignancies, the potential biologic effects of consumer powders (with their variable constituents) on the human ovary have not been well studied.

The prevalence of genital powder exposure reported among control women in this and other studies conducted in the United States ranges from 28 percent to 51 percent (4–6, 8, 10). Given such a common practice, even the modest elevation in ovarian cancer risk associated with genital powder application suggested by most of the epidemiologic studies could have a notable impact on the incidence of ovarian cancer in the United States. We recommend that cohort studies address this question; these studies could eliminate concerns regarding the potential differences in the reporting of genital powder exposures between cases and controls. We also believe that further characterization of the constituents of powder products that may influence ovarian cancer risk and the investigation of their possible biologic mechanisms of carcinogenesis are warranted.

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REFERENCES

1. Egli GE, Newton M. The transport of carbon particles in the human female reproductive tract. Fertil Steril 1961;12:151–5.
2. Ventor PF, Iturralde M. Migration of a particulate radioactive tracer from the vagina to the peritoneal cavities and ovaries S Afr Med J 1979;55 917–19
3. Henderson WJ, Joslin CA, Turnbull AC, et al Talc and carcinoma of the ovary and cervix J Obstet Gynaecol Br Commonw 1971;78 266–72.
4 Cramer DW, Welch WR, Scully RE, et al. Ovarian cancer and talc· a case-control study. Cancer 1982;50:372–6
5. Hartge P, Hoover R, Lesher LP, et al. Talc and ovarian cancer. (Letter). JAMA 1983,250:1844.
6 Whittemore AS, Wu ML, Paffenbarger RS Jr, et al. Personal and environmental characteristics related to epithelial ovarian cancer. II Exposures to talcum powder, tobacco, alcohol, and coffee. Am J Epidemiol 1988;128:1228–40
7 Booth M, Beral V, Smith P Risk factors for ovarian cancer a case-control study. Br J Cancer 1989;60:592–8.
8 Harlow BL, Weiss NS A case-control study of borderline ovarian tumors: the influence of perineal exposure to talc. Am J Epidemiol 1989,130:390–4.
9. Chen Y, Wu PC, Lang JH, et al. Risk factors for epithelial ovarian cancer in Beijing, China. Int J Epidemiol 1992,21· 23–9.
10 Harlow BL, Cramer DW, Bell DA, et al Perineal exposure to talc and ovarian cancer risk Obstet Gynecol 1992,80·19–26.
11 Rosenblatt KA, Szklo M, Rosenshein NB. Mineral fiber exposure and the development of ovarian cancer. Gynecol Oncol 1992;45 20–5
12. Harlow BL, Hartge PA. A review of perineal talc exposure and risk of ovarian cancer. Regul Toxicol Pharmacol 1995; 21.254–60.
13. Waksberg J Sampling methods for random digit dialing. J Am Stat Soc 1978,73·40–6.
14. Breslow NE, Day NE, eds. Statistical methods in cancer research. Vol 1. The analysis of case-control studies Lyon: International Agency for Research on Cancer, 1980. (IARC scientific publication no. 32)
15. World Health Organization. International classification of diseases for oncology. 2nd ed Geneva: World Health Organization, 1990.
16 Cosmetic, Toiletry, and Fragrance Association (CTFA). Talc specification and methods. In CTFA compendium of cosmetic ingredient composition. Washington, DC: CTFA, 1976 10–17
17 Cralley LJ, Key MM, Groth DH, et al. Fibrous and mineral content of cosmetic talcum products Am Ind Hyg Assoc J 1968;29·350–4
18 Rohl AN, Langer AM, Selikoff IJ, et al Consumer talcums and powders: mineral and chemical characterization J Toxicol Environ Health 1976;2 255–84
19. Zazenski R, Ashton WH, Briggs D, et al. Talc· occurrence, characterization, and consumer applications. Regul Toxicol Pharmacol 1995;21:218–29.
20 Tzonou A, Polychronopoulou A, Hsieh C, et al Hair dyes, analgesics, tranquilizers, and perineal talc application as risk factors for ovarian cancer Int J Cancer 1993;55:408–10
21. Kasper CS, Chandler PJ. Possible morbidity in women from talc on condoms JAMA 1995,273·846–7.
22 Paoletti L, Caiazza S, Donelli G, et al Evaluation by electron microscopy techniques of asbestos contamination in industrial, cosmetic, and pharmaceutical talcs. Regul Toxicol Pharmacol 1984;4:222–35
23. National Toxicology Program (NTP) Toxicology and carcinogenesis studies of talc in F344/N rats and B6C3F$_1$ mice (inhalation studies). Research Triangle Park, NC. US Department of Health and Human Services, 1993. (NIH publication no. 93–3152).
24 Roggli VL. Human disease consequences of fiber exposures· a review of human lung pathology and fiber burden data Environ Health Perspect 1990,88:295–303.
25 Weiss NS, Cook LS, Farrow DC, et al Ovary. In: Schottenfeld D, Fraumeni JF, eds Cancer epidemiology and prevention. 2nd ed. New York: Oxford University Press, 1996.